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RESPONSE OF THE HYPERTHYROID HEART TO EPINEPHRINE

C. H. McDONALD, WALTER LEE SHEPEARD, M. F. GREEN AND A. F. DeGROAT

From the Department of Physiology and Pharmacology, University of Arkansas, School of Medicine, Little Rock

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The effect of thyroid substance or of thyroxine upon tissues is the subject of much investigation the results of which are not always in agreement. Kalnins (1928) reported no increase in the effect of epinephrine upon the excised frog heart following perfusion with a solution containing thyroxine; he reported a slightly lower heart rate with increased amplitude of beat following such perfusion. Paasch and Reinwein (1928), Hopping (1931) and others found no increase in tissue metabolism from thyroxine. Davis, Da Costa and Hastings (1934) have recently demonstrated a marked but delayed increase in the metabolism of the isolated frog heart on perfusion with thyroxine. Their work does not reveal whether this increased metabolism is due to an increased beat of the heart.

Prolonged administration of thyroid has generally shown an increased activity of structures innervated by the sympathetic system. A tachycardia (MacIntyre, 1931), an augmented response to epinephrine by the iris (Bergwall and Kuchinsky, 1931), an augmented blood pressure and heart rate (Santesson, 1919; Lütolf, 1930), and a restoration to normal metabolism, heart rate, and response to epinephrine in thyroidectomized cats (Sawyer and Brown, 1935) are described following administration of thyroid or thyroxine. It is theorized that thyroid sensitizes the structures upon which epinephrine acts.

This series was designed to study simultaneously the rate of beat, the rate of oxygen consumption, and the response to epinephrine of the isolated heart of terrapins made hyperthyroid by the prolonged administration of desiccated thyroid perorally. The apparatus employed and the manner of perfusion were described by one of us (McDonald and McDonald, 1935).

A suspension of desiccated thyroid substance[1] in water was placed in a small hypodermic syringe fitted with a no. 13 Fr. catheter. The terrapin's head was withdrawn from its shell, its jaws held open with a screw clamp, the catheter introduced as a stomach tube, and sufficient of the suspension injected to equal 0.001 gram for each 10 grams of body weight as the initial dose; later dosage was 0.001 gram for each 50 grams of body weight. The animals were given thyroid substance once each week for a period of four weeks. There was an early loss of weight, a diarrhea with yellowish, mucus-laden stools. A muscular weakness appeared after two or three weeks; if this weakness was marked or developed earlier than the average the dosage was cut down or omitted for a week. We lost four terrapins by death.

A pronounced tachycardia was accepted as evidence of a hyperthyroid state. Only 1 of 18 terrapins to which thyroid was administered failed to respond with a heart rate much more rapid than that exhibited by the controls. This heart was discarded as were those of two controls which exhibited rates substantially more rapid than that which we have observed to be average for the isolated terrapin heart. The perfusion fluid in all experiments was of the following composition:

	per cent
Sodium chloride	0.650
Potassium chloride	0.014
Calcium chloride	0.012
Sodium bicarbonate	0.020
Sodium dihydrogen phosphate	0.001

Observations were for periods of one hour unless otherwise indicated; the hearts were then laid open, blotted dry, and weighed. The oxygen consumed was reduced to standard conditions and calculated in cc./gm./hr. Table 1 sets forth, in averages, the rate of oxygen consumption and of beat of the isolated hearts of controls and of terrapins made hyperthyroid through prolonged peroral administration of thyroid.

Table 2 sets forth, in averages, the rate of oxygen consumption, rate of beat, and response to epinephrine of control and hyperthyroid hearts. These were perfused with the Ringer's solution for 30 minutes, observations being made upon the rate of oxygen consumption and rate of beat; epinephrine-HCl solution was then added to the perfusing fluid to the concentration of 1:500,000. The point of introduction of the epinephrine into the apparatus delayed for a short time a response by the heart. Maximum increase in the rate of beat was observed to occur at an average of 3 minutes following introduction of the epinephrine; the rate at this time was selected

[1] The desiccated thyroid substance was furnished through the courtesy of Parke, Davis & Co.

as the standard response in rate of beat to epinephrine. The oxygen consumption was determined for a period of 30 minutes following the introduction of epinephrine.

Acting upon the theory that the tachycardia of hyperthyroidism is due to a sensitization of the structures upon which epinephrine acts, we attempted to paralyze the sympathetic endings in the isolated heart through

TABLE 1

A comparison of the rate of oxygen consumption and the rate of beat of control terrapins and hyperthyroid terrapins

TYPE	NUMBER OF EXPERIMENT	O_2 CONSUMPTION	HEART RATE		INCREASE O_2 CONSUMPTION	RATE OF BEAT
			Initial	Final		
		cc./gm./hr.			*per cent*	
Control	6	1.57	27	25		
Hyperthyroid	10	2.76	44	40	75	63

TABLE 2

A comparison of the response to epinephrine by the isolated hearts of control terrapins and hyperthyroid terrapins

TYPE	NUMBER OF EXPERIMENT	O_2 CONSUMPTION		HEART RATE		INCREASE IN RATE FOLLOWING EPINEPHRINE	
		Ring.	Epin.	Ring.	Epin.	O_2 consumption	Beat
		cc./gm./hr.				*per cent*	
Control	6	1.60	2.33	32	38	46	18.7
Hyperthyroid	6	3.08	6.98	46	56	126	21.7

TABLE 3

A comparison of the effect of ergotoxine upon the isolated hearts of control and of hyperthyroid terrapins

TYPE	NUMBER OF EXPERIMENT	HEART RATE			
		Initial →	Ergotoxine →	Epineph. →	Ergotoxine
Control	24	34	31	40	36
Hyperthyroid	2	48	34.5	46	48

the use of ergotoxine ethanesulphonate;[2] 0.5 gram of the substance was dissolved in 150 cc. of the perfusion fluid and recirculated through the heart several times. Alterations in the rate of contraction and in the response to epinephrine of these hearts are shown in table 3. Because of

[2] Ergotoxine ethanesulphonate furnished through courtesy of Eli Lilly & Co. and Abbott Laboratories.

reduction in the surface tension with slight frothing of the circulating medium, oxygen consumption observations were invalidated.

DISCUSSION. It is obvious from these experiments that prolonged feeding of thyroid to terrapins results in a tachycardia and an increased consumption of oxygen by the heart. The rate of increase in oxygen consumption parallels the rate of increase in heart beat close enough that when the factor of experimental error is taken into account there appears to be little, if any, increase in amplitude of beat as a result of prolonged administration of thyroid. In the response to epinephrine the hyperthyroid hearts showed a slightly greater increase in rate of beat and a much greater increase in rate of oxygen consumption than did the controls. The greater increase in amplitude and force of contraction in the hyperthyroid hearts following epinephrine is easily observable; whether these factors wholly account for the increased rate of oxygen consumption is not determined by these experiments.

SUMMARY

1. Prolonged feeding of thyroid to terrapins results in a tachycardia and an increased rate of oxygen consumption by the isolated heart.

2. It leads to an increased response to epinephrine; the increase in rate of beat is slight, that in rate of oxygen consumption is enormous as compared to the same response by controls.

3. If ergotoxine exerts any paralyzing effect upon the sympathetic structures within the heart in the dosage employed in these experiments, this effect is abolished by the action of epinephrine, both in the control heart and in the hyperthyroid heart.

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A STUDY IN REFLEXES: IDENTIFICATION OF THE CUTANEOUS AFFERENT FIBERS WHICH EVOKE IPSILATERAL EXTENSOR AND FLEXOR REFLEXES

A. SIDNEY HARRIS

From the Physiological Laboratory of Washington University School of Medicine, Saint Louis

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The application of the cathode ray oscillograph to the study of nerve action potentials has in recent years provided criteria whereby the active fibers in a nerve may be differentiated in terms of fiber diameter. These criteria are 1, electrical threshold, and 2, rate of conduction of the impulse (Erlanger and Gasser, 1924; Gasser and Erlanger, 1927). By applying these criteria, Heinbecker, Bishop and O'Leary (1933, 1934) demonstrated a specificity of groups of afferent fibers for the skin senses: touch, pain, warmth and cold, and recognized the band that each occupies in the fiber spectrum. The employment of the above mentioned criteria in this study has demonstrated that qualitatively different reflex responses are evoked by relatively discrete groups of afferent fibers as characterized by diameter, irritability, and conduction rate, but there is overlapping of the ranges that they occupy.

In the early experiments, exploratory trial stimulation of various large nerves and posterior roots of the spinal bullfrog (*Rana catesbiana*), while registering the reflex contractions in ipsilateral extensor and flexor muscles of the hind limb, yielded variable and confusing results. The variations were similar to those reported by earlier workers as, for example, Graham Brown (1911), and Sherrington and Sowton (1911a and b). Usually the lowest threshold response was that of ipsilateral extension, sometimes flexion, and at other times both would appear simultaneously, or one quickly after the other without alteration of the applied stimuli. It appeared quite impossible to work out from the results of stimulation of large mixed nerves any system of differentiation by thresholds which would relate the type of response to any limited group of afferent fibers.

Further exploration revealed that stimulation of the skin posterior to the gastrocnemius muscle gave in many cases a reflex of apparently pure ipsilateral extension. Search of the literature then showed that not only did stimulation of this area yield primarily extension reflexes, but the same was also true of the skin in the region of the Achilles tendon, the plantar

surface, and over the triceps femoris muscle. The stimulation of certain other areas results wholly or chiefly in flexion. This is true of the skin of the toes, the dorsum of the foot, and the front of the leg. Light touch constitutes an adequate stimulus for either of these responses when applied to an appropriate area. Figure 2 is a record of extensor and flexor reflex contractions recorded from the triceps and semitendinosus muscles respectively, showing the specificity of localization of the areas from which the responses may be obtained. The stimulus used in this experiment was light stroking of the skin with a wet camel's hair brush. Light pinching with forceps, or weak electrical stimulation of the skin gives similar results.

None of the authors (Beritoff, 1913, 1923; Fröhlich, 1909; and Baglioni, 1904) who have reported on the reflexes evoked by stimulation of different skin areas dissected the nerves free from the skin and stimulated them directly, nor did they study their fiber composition. The cutaneous nerves of the leg and foot of the bullfrog were used in the experiments to follow.

METHODS. *Method of comparing the thresholds of the most irritable fibers in different nerves.* In order to be able to correlate reflex thresholds, found under conditions to be described later, with nerve fibers of any given irritability group it was necessary to have for comparison some standard of reference. As the most dependable and available standard the threshold of the most irritable motor fibers in some suitable nerve, as indicated by the contraction of the muscles innervated by the nerve, was chosen. Sherrington (1894) showed that the largest fibers in the nerves of the hind limb of the mammal are motor. Erlanger et al. (1926, 1927) demonstrated with the cathode ray oscillograph that the motor fibers are among the most irritable and most rapidly conducting fibers in the hind limb nerves of both the dog and frog. No differences in irritability or conduction rate were found between the most irritable motor and the most irritable sensory fibers.

When the reflex responses occurring upon stimulation of a cutaneous nerve are being studied, the same nerve obviously cannot be used as a part of a nerve-muscle preparation for comparison. Since it was necessary, therefore, to compare with another nerve it was essential to find a system by which it would be possible to make valid comparisons of the threshold of fibers in one nerve with that of fibers in another. There are two factors which qualify comparison, *a*, secondary resistance when stimulating with induced currents, and *b*, differences in the amount of shunting by inactive tissue (when stimulating by any method). Both differences are reduced relatively by an external shunt. For this purpose, use was made of a low resistance potential divider which was also employed to grade the stimulating voltage applied to the nerve. When this system was used the changes in threshold when the point of stimulation was moved from a large part to a small part of the nerve were no larger than the random variations at a point, namely, 2 to 4 per cent.

In the early experiments stimulation was accomplished by an inductorium operated by a rotating interrupter in the primary circuit. With high frequencies, however, this method is not entirely satisfactory due to possible chattering of keys and to foreshortening of the make shock which is believed to be short circuited (Erlanger and Garrey, 1914). Therefore a thyratron stimulator (see Schmitt and Schmitt, 1932) was employed in all high frequency experiments (60 to 120 per second, and sometimes higher). This stimulator is free from both of the difficulties which beset the other type of apparatus as it does not involve the use of any keys or short circuits.

In almost all of the experiments from which threshold data are reported, contact with the nerve was achieved through mercury-calomel non-polarizable electrodes. The glass tips of the electrodes were made in such a way that a small nerve could be suspended across them, making contact by lying in a meniscus of the calomel saturated Ringer's solution which filled the cells.

Three methods of attack were employed in the study: 1. The nerves were stimulated electrically and their qualitative reflex responses and the thresholds of these responses were determined. The reflex thresholds were compared with the motor fiber threshold of the peroneal nerve low in the leg. It was found that the peroneal

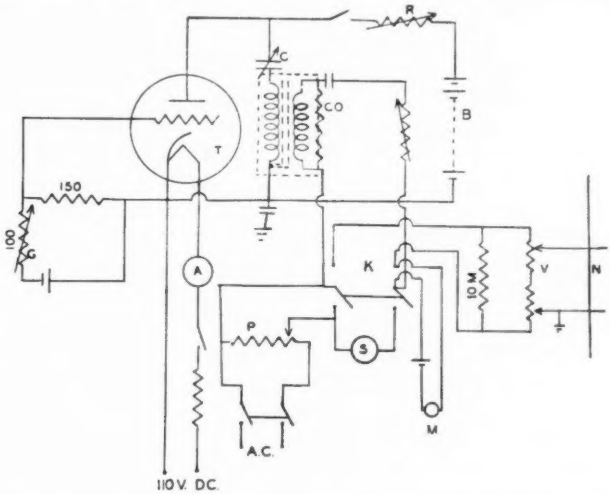


Fig. 1. Thyratron stimulator circuit with mechanisms for calibration of frequency, and for recording the time of closing the stimulating circuit.

V, 412 ohm potential divider mentioned in the text; *T*, FG 67 thyratron tube; *G*, 100 ohm variable resistance to vary the grid bias; *C*, variable condenser which discharges through the tube when the breakdown potential is reached; *R*, variable resistance used to regulate the frequency of oscillation; *CO*, shielded coreless Porter inductorium with 10,000 ohm resistance across the secondary terminals; *K*, double pole double thrown switch which may be thrown in one direction to close the stimulating circuit, or in the other to close the calibrating circuit; *S*, magnetic loud speaker used for calibration of frequency; *P*, potential divider in 60 cycle A.C. line used for calibration; *M*, signal magnet to mark the time of closing the stimulating circuit.

threshold is approximately identical with that of tibialis superficialis as indicated in each case by the contraction of toe muscles. The threshold of the most irritable fibers in either of these nerves is slightly lower than that of the fibers in the triceps nerve which was used first. It was thought desirable to make the comparisons with a motor nerve containing the most irritable fibers from the sciatic. 2. The diameters of the large fibers in the various nerves were measured from histological preparations. 3. The thresholds and conduction rates of the fibers composing the various cutaneous nerves studied were determined with the cathode ray oscillograph.

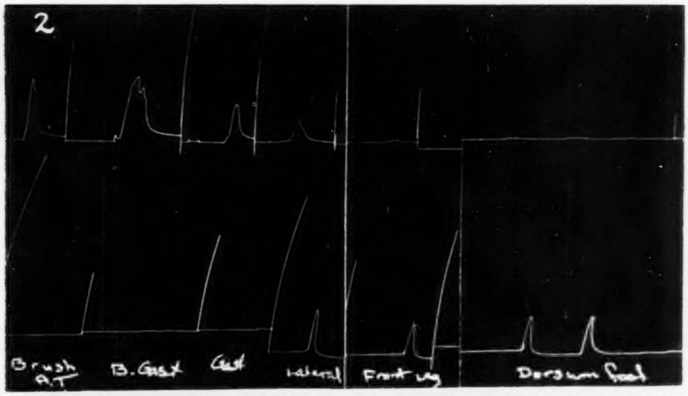
These were compared with the thresholds and conduction rates of their parent tibial and peroneal nerves. In each case the sciatic was stimulated and the leads to the amplifier taken from the cutaneous branch being studied, or from a distal portion of the parent trunk near the point of departure of the cutaneous branch. After the nerve was placed on the electrodes, it was not disturbed until all determinations on both the branch and parent trunk were completed.

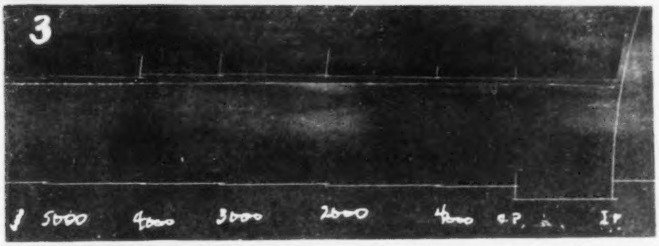
QUALITATIVE REFLEX RESPONSES TO STIMULATION OF THE VARIOUS NERVES. *Ramus cutaneus cruris posterior* (*posterior nerve*). Since stimulation of the skin posterior to the gastrocnemius muscle gave as its usual lowest threshold response ipsilateral extension, it was reasoned that stimulation of the nerve supplying this area should evoke a similar response. This nerve, the posterior, is a branch of the tibial. With care about 3 or 4 cm. may be dissected free from the skin. The reflex response was observed in two ways; first, by recording the activity of the triceps and semitendinosus muscles on a smoked paper kymograph; and second, by watching the contraction in the triceps. It was found that simply watching for threshold responses was quite as sensitive as kymographic registration. The smallest threshold contraction usually occurs in the same part of the muscle in all preparations, appearing in the lateral-distal part near the patellar tendon.

The one response to stimulation of the posterior nerve which invariably occurred was that of ipsilateral extension as expressed by contraction of the triceps and gastrocnemius muscles. The triceps always responded more vigorously than did the gastrocnemius. In some animals it was possible also to obtain flexor reflexes upon stimulation of this nerve, but the two types of responses were easily separable in terms of the threshold intensities of the shocks required to evoke them, and the vigor of the responses.

Frequently, ipsilateral extension was the only response obtainable by stimulation of this nerve, and in such cases the semitendinosus usually showed reflex relaxation. In some experiments the intensity of stimulation was carried to more than one hundred times the extensor reflex threshold without changing the character of the response. If flexion appeared at all, it began at about two to two and a half times the threshold for the extensor reflex. In those experiments in which no flexor contraction occurred, the extensor response was as sensitive and appeared to be as strong as in other experiments in which a flexor contraction did occur. The flexor excitation was frequently manifest only as post inhibitory rebound as in figure 4.

Figure 3 shows a record of pure ipsilateral extension accompanied by flexor relaxation. In order to test whether or not the flexor reflex central apparatus was functional a toe of the ipsilateral foot was lightly pinched. The semitendinosus responded with a vigorous contraction. It would seem from these experiments that one of two conditions must prevail;





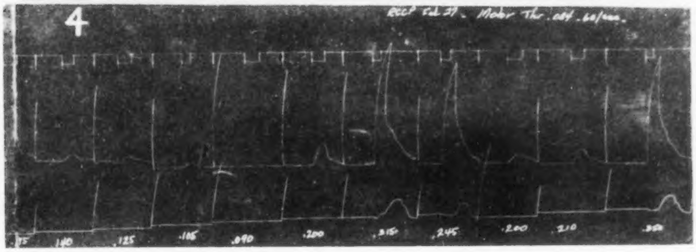


Fig. 2. Reflexes elicited by touching the skin with a wet camel's hair brush.

Upper line, triceps. Lower line, semitendinosus.

Left to right, touch over the Achilles tendon, posterior to the gastrocnemius muscle (two records), lateral to the gastrocnemius, front of the leg and the dorsum of the foot (two records).

Fig. 3. Reflex responses to stimulation of the posterior nerve.

Upper line, triceps; lower line, semitendinosus.

Each record made with the drum stationary.

Figures, ohms in the primary circuit.

C.P., pinch of toe of contralateral foot. *I.P.*, pinch of toe of ipsilateral foot.

Fig. 4. Reflex responses to stimulation of the posterior nerve 3 or 4 hours after transection of the spinal cord showing flexor post-inhibitory rebound contractions.

Upper myogram, triceps; lower myogram, semitendinosus.

The numbers indicate potential divider readings, proportional to the applied voltage.

either the posterior nerve contains no fibers which mediate the flexion reflex, or the center for flexion was so greatly inhibited by the reciprocal innervation of the extensor mechanism that the flexion reflex failed to appear. Not only did the flexor muscle fail to respond by contraction, but it relaxed, indicating that its previous tonic discharge was diminished. The first of the conditions postulated above cannot be true, because in some experiments a flexion reflex is obtained upon stimulation of this nerve, the threshold for this response being high as previously mentioned. Therefore, the other alternative must hold: the flexor muscles did not respond because their center was so greatly inhibited.

Nervus cutaneus dorsi pedis lateralis (dorsal nerve). Stimulation of this nerve which is a branch of the peroneal supplying the skin of the dorsum and dorsolateral part of the foot gives flexion as practically its only reflex response. It is true that brushing the dorsolateral region evokes a mixed response, but with the microscope almost all of the nerve filaments from this area can be traced into the tibial nerve, not into the dorsal. However, in some of the records of reflex contractions evoked by stimulation of this nerve the triceps record shows a barely perceptible rise above the base line while the flexor muscle responds with a vigorous contraction. In other records, there is no trace of a response in triceps. Certainly one can say without hesitation that almost all of the response is in the flexor muscle.

In these experiments, the hip flexor component of triceps was denervated. Inspection of the triceps muscle during an extensor reflex contraction, and during a flexor reflex contraction shows clearly that these two responses in triceps occur in different parts of the muscle, each in its own end. The distal half contracts during the extensor reflex, and the proximal part during flexion. The innervation of triceps is likewise in two parts; part by way of the crural nerve, and part by way of the triceps nerve from the sciatic. The crural nerve apparently is distributed only to the flexor end of the muscle. The triceps, however, is divided into branches, chiefly two, the largest of which courses distally to the extensor end, and the smaller branch courses to the proximal part. By cutting the crural, and the proximal branch of the triceps nerve it is possible to denervate the flexor part of the muscle, perhaps completely, without interfering with the nerve supply to the extensor end.

Figure 5 shows the responses which may be obtained upon stimulation of the dorsal nerve with the flexor part of triceps denervated. In A the rises in the extensor line are slight, but the flexor contractions are quite strong. B is from another experiment. The extensor reflex was shown to be functional by stimulating the skin posterior to the gastrocnemius muscle. A strong contraction in the extensor end of triceps resulted.

It might be argued that the slight activity in the extensor record is in reality flexor, and due to an incomplete denervation of the flexor com-

ponent. The fact remains, however, that the small rise in the extensor line occurred at a lower threshold than did the contraction in semitendinosus. This indicates that it is unlikely to be a flexor response. The extensor response to stimulation of this nerve, however, is extremely small if it occurs, and the primary reflex response to stimulation of the dorsal nerve is flexion.

Ramus cutaneus medialis inferior (*medial nerve*). This nerve is double, being in reality two branches from the tibialis superficialis. They leave the parent trunk near the same level, slightly below the middle of the gastrocnemius muscle, course distally and supply the skin in the region of and medial to the Achilles tendon. The two branches supply contiguous areas, and are similar in the reflex responses that they evoke. They are similar also in each of their other properties studied. The reflex response to stimulation of these nerves is qualitatively similar to that found in the case of the posterior.

The primary response is, therefore, ipsilateral extension at low threshold and the addition of flexion at higher threshold. One difference is to be noted: though one always obtains extension as the lowest threshold response, the flexion which appears when the intensity of stimulation is sufficiently raised is more vigorous than in the case of the posterior nerve, and appeared in all of the experiments. This would seem to mean that these nerves contain a larger proportion of fibers which mediate the flexor reflex than does the posterior.

Ramus cutaneus plantae lateralis (*plantar nerve*). This also is a branch of tibialis superficialis. It leaves the parent trunk at the level of the plantar aponeurosis and supplies the skin of the sole of the foot. Though it gives as its lowest threshold response ipsilateral extension, the responses are somewhat different in character from those of other nerves studied. Before dissection of the leg, stimulation of the skin of the sole gives rise to a powerful bilateral extensor thrust. This response was described by Baglioni (1904). After the nerve is dissected free and the muscles prepared for the registration of reflex response s,stimulation of the nerve results in a low threshold extensor contraction as shown in figure 7 but it has lost the nature of a thrust. Flexion appears also at about 1.8 to 2 times the extensor threshold and upon the use of strong shocks it becomes very powerful in contrast to the weak reaction inhibited almost to extinction in the case of the posterior nerve.

The extensor thrust occurs first and more powerfully in the hip extensor muscles, followed by contraction of the knee extensors. Efforts have been made to register it both from the gracilis major and triceps muscles, but in no case has it occurred after dissection and fixation. Records of the thrust were made by inserting a pin into the patellar tendon through a small hole in the skin and recording the extensor movement of the whole thigh.

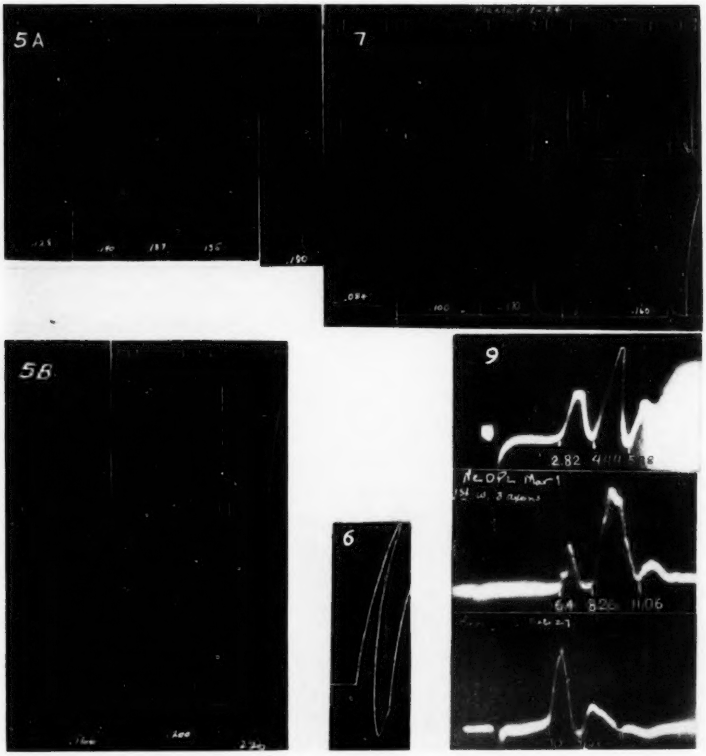


Fig. 5 A and B. Reflex responses to stimulation of the dorsal nerve. Records from two experiments. In each record: upper myogram, triceps and lower myogram, semitendinosus. Record A shows a trace of extensor response in addition to the flexor contractions and at a lower threshold. Record B shows flexor contraction only. Numbers as in figure 4.

Fig. 6. Extensor thrust evoked by stimulating the skin of the plantar surface. Record of movement of the leg, attachment being made to the patellar tendon over the knee.

Fig. 7. Reflex responses to stimulation of the plantar nerve. Upper myogram, triceps. Lower myogram, semitendinosus. Numbers as in figure 4.

Fig. 9. Action potential records from the posterior nerve (upper), dorsal nerve (middle) and the peroneal (lower).

The numbers under the records indicate the time in ms. from the delivery of the shock to the beginning of the elevation designated.

The conduction distances were: posterior 75.7 mm.; dorsal 135 mm.; and peroneal 96 mm.

All records were made with linear time axis.

The skin of the plantar surface was stimulated by rapidly repeated shocks. Figure 6 shows the contraction.

Ramus cutaneus cruris lateralis (*lateral nerve*). This nerve is a branch of the peroneal. It leaves the parent trunk just above the knee and courses downward and laterally supplying the skin covering the lateral part of the crus. When it is stimulated with shocks of suitable intensities, responses are evoked in both the extensor and flexor muscles, the flexor response being the greater when maximal. The extensor response, however, occurs at a lower threshold than does the flexor. Threshold relationships will be set forth more definitely in the section on thresholds.

Reflex thresholds. In order to obtain reflex responses by stimulation of as few afferent fibers as possible, i.e., to cause the reflex threshold to approach the threshold of the most irritable afferent fibers which mediate the reflex, it is essential to use repetitive stimulation. This is a necessary corollary to the findings of Eccles and Sherrington (1930). They reported that shocks of a certain intensity which singly will not evoke a reflex response are adequate to elicit a reflex contraction if sent in in pairs, the two shocks being separated by a suitable interval. They found that for the flexor reflex in the cat the two shocks are most effective when separated by 6 to 8 milliseconds (ms.). This interval would correspond to a frequency of 125 to 167 per second. A single impulse arriving at the center by way of an afferent nerve fiber will not excite the center sufficiently to cause a reflex response. An excitatory condition (central excitatory state) must be built up to neuron threshold before there is any discharge from the motoneurones. This can be done in either of two different ways: 1, by a single shock applied to the nerve if the intensity of the shock is great enough to excite a sufficiently large number of fibers that are concerned with the reflex, or 2, by exciting one or a few fibers at a sufficiently rapid rate. In the latter case, when the optimally effective frequency[1] is used, the reflex threshold approaches or becomes the same as the threshold of the most irritable fiber which serves to elicit the reflex. This optimum is approached gradually as the frequency is increased from single shocks or a low beginning frequency. As the frequency increases, within the range below the optimum, the reflex threshold intensity decreases.

The accompanying graph, figure 8, shows the relation that exists between frequency of stimuli and threshold intensity for the ipsilateral extensor reflex evoked by stimulation of the posterior nerve, and for the flexor reflex evoked by stimulation of the dorsal. The curves show that the optimally effective frequency for the extensor reflex is reached at about 120 per second while that for the flexor reflex is about 60. This agrees

[1] The optimally effective frequency means that frequency beyond which further increases fail to further lower the reflex threshold.

very well with the optimal frequency-tension data of Sassa (1921) for the flexor reflex in the frog.

At the optimally effective frequency the extensor reflex threshold intensity becomes practically identical in value with that of the most irritable motor fiber in the peroneal nerve which was used for comparison, whereas that of the flexor reflex remains about 1.6 to 1.7 times as high.

The excitation threshold of nerve fibers, as shown by stimulating a motor nerve and observing the resulting muscular contractions, does not decrease with increasing frequency of stimulation within the range used,

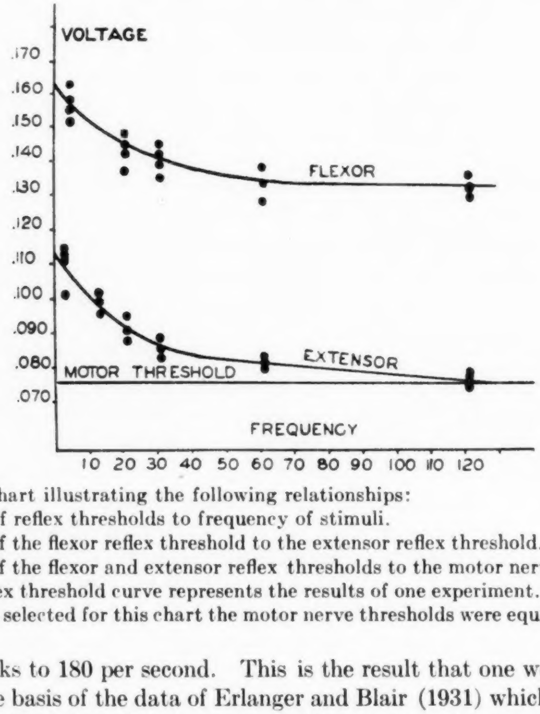


Fig. 8. Chart illustrating the following relationships:
1. That of reflex thresholds to frequency of stimuli.
2. That of the flexor reflex threshold to the extensor reflex threshold.
3. That of the flexor and extensor reflex thresholds to the motor nerve threshold.
Each reflex threshold curve represents the results of one experiment. In the two experiments selected for this chart the motor nerve thresholds were equal.

single shocks to 180 per second. This is the result that one would anticipate on the basis of the data of Erlanger and Blair (1931) which show that the summation interval in large nerve fibers at room temperature is only about 0.2 to 0.6 ms. Very much higher frequencies than any used in this study would have to be employed in order to lower the threshold of the fibers. The depression phase following a subthreshold shock is of much longer duration, being of the order of 3 to 5 ms. The depression phase is similar in this respect to the relatively refractory period following the discharge of an impulse. The real danger in the use of high frequencies, therefore, would be that of sending in shocks during the period of depression

and finding a higher threshold. In view of the data cited, this does not become a factor unless stimuli more frequent than 200 per second are employed. The frequencies used in the experiments here reported usually have not been higher than 120 per second.

The reflex threshold could never approach the motor nerve threshold unless the most irritable afferent fiber which serves to evoke the reflex is as irritable as the lowest threshold motor fiber in the nerve which is used for comparison. The reflex threshold data and the motor nerve threshold data contained in table 1 are typical of those found for the various nerves that have been studied.

TABLE 1

Reflex and motor nerve thresholds

NERVE	EXTENSOR THRESHOLD	FLEXOR THRESHOLD	MOTOR THRESHOLD
Posterior 1-15	0.073 (60 sec.) 0.069 (120 sec.)	No response	0.068
Posterior 1-16	0.078 (60 sec.) 0.075 (120 sec.)	No response	0.075
Posterior 2-10	0.090 (60 sec.) 0.084 (120 sec.)	0.178	0.082
Posterior 2-27	0.082 (60 sec.)	0.147	0.077
Dorsal 2-27	None (60 sec.)	0.140	0.084
Dorsal 1-20	None (60 sec.)	0.128	0.082
Dorsal 1-22	None (60 sec.)	0.124	0.074
Dorsal 3-14	0.128 (60 sec.) (trace)	0.135	0.084
*Medial 1-24	0.042 (60 sec.)	0.080	0.040
Medial 1-26	0.083 (60 sec.)	0.167	0.078
*Plantar 1-24	0.048 (60 sec.)	0.080	0.044
Lateral 3-20	0.100 (60 sec.) 0.096 (120 sec.)	0.110 0.111	0.094
Lateral 3-23	0.085 (60 sec.) 0.080 (120 sec.)	0.129 0.129	0.075 0.075

* Made with silver electrodes. All other determinations made with calomel electrodes.

The thyratron stimulator was used in all of the experiments listed above. The values are expressed in arbitrary units of the applied potential.

A further significant fact which one may glean from the table is that the threshold of the flexor reflex is higher if it is evoked from a nerve whose main response is extension than if evoked by stimulating a nerve whose main response is flexion. Table 2 will show this more clearly. This difference is, no doubt, significant and is probably to be explained as due to the inhibitory influence exerted by the extensor afferent fibers upon the center for flexion. It is to be recalled in this connection that in the myo-

grams registering the movement of the triceps and semitendinosus muscles when the posterior nerve is stimulated one frequently sees only flexor relaxation accompanying extensor contraction. When the flexor does contract the contraction is usually preceded by a phase of relaxation, the relaxation occurring simultaneously with the extensor contraction.

The truer values for the flexor reflex threshold, therefore, are no doubt those found by stimulation of a nerve such as the dorsal which does not evoke any appreciable extensor contraction. The most irritable fibers which evoke the flexion reflex then are fibers whose thresholds are 1.6 to 1.7 times the threshold of the largest motor fibers.

OBSERVATIONS MADE WITH THE CATHODE RAY OSCILLOGRAPH. *Thresholds and conduction rates.* By the use of the cathode ray oscillograph to study the action potentials of the various nerves the following types of data were obtained: 1. The thresholds of the most irritable fibers and of those con-

TABLE 2

Ratio of flexor reflex threshold to motor threshold

EXTENSOR NERVE		FLEXOR NERVE	
Posterior	2.17	Dorsal	1.66
	1.90		1.56
	2.92		1.68
			1.70
Medial	2.17		1.63
	2.14		1.85
			1.69
Plantar	2.17		1.62
Average	2.24		1.67

tributing to the various elevations in the action potential record both of the cutaneous nerve and the parent trunk have been determined; 2, the conduction rates of the fastest conducting fibers in the nerve and the first fibers contributing to the various elevations have been ascertained; and 3, the configuration of the action potential wave complex has been observed. In tables 3 and 4 certain relationships derived from the findings are summarized.

Configuration of the action potential records. Figure 9 reproduces in reduced size the action potential records of the posterior, dorsal and peroneal nerves. Both in height and area, the first elevation of the posterior nerve action potential is greater than that of the dorsal with respect to the second elevation, or the second elevation of the dorsal is relatively the greater. The ratios of heights and areas of the first two elevations as measured from the original pictures are shown in table 5.

By counting the single axon action potentials as they appeared on the

screen of the oscillograph while the strength of stimulation was gradually being increased, it was seen that only three axons contributed to the first elevation of the dorsal while about 12 to 15 axon potentials could be identified as they joined the first elevation of the posterior. Microscopically, these figures approximately correspond to the number of fibers more than 10 μ in diameter in each of the nerves.

TABLE 3

Ratio of elevation thresholds to the threshold of the first elevation of the parent nerve

CUTANEOUS CURVE		ELEVATIONS				PARENT NERVE	ELEVATIONS			
		1	2	3	4		1	2	3	4
Post.	1–17	1.00	1.88	3.71	22.66	Tib.	1.00			
Post.	1–10	1.07	1.88	3.13	10.34	Tib.	1.00			
Med. 1	2–8	1.00	1.73	3.42	5.87	Tib. S.	1.00	1.63	2.85	8.00
Med. 2	2–8	0.98	1.59	2.10	8.00	Tib. S.	(same nerve)			
Plant	2–23	1.00	1.77	4.50	10.40	Tib. S.	1.00			
Averages......		1.01	1.77	3.37	11.45					
Lat.	3–20	1.45	2.30	5.44		Per.	1.00	2.22		
Lat.	3–23	1.07	1.48	2.60	5.20	Per.	1.00	1.56	2.52	
Dors.	3–1	1.30	1.53	2.69	5.80	Per.	1.00	1.67		
Dors.	3–14	1.38	1.60	2.35		Per.	1.00	1.56	2.52	
Dors. averages.........		1.34	1.57	2.52	5.80					
All parent nerve averages taken together..............							1.00	1.73	2.66	8.00

The data on the lateral nerve are separated from the others, and not included in the averages because of the fact that this nerve supplies a transitional area of skin, and exhibits greater variations than do the other nerves.

In all of the nerve action potentials, one frequently sees a few scattered axon potentials which do not form a real elevation. This is especially true beyond the third elevation. It is, therefore, sometimes difficult to decide just what group of potentials to count as an elevation. This is the reason for the large variation in the figures listed for the fourth.

The tables show that the threshold of the most irritable fiber in this particular posterior nerve was 1.07 times that of the most irritable fiber in the parent trunk. This was the highest ratio found for any posterior nerve, the more usual ratio being 1.00. The threshold of the most irritable fiber in the dorsal nerve whose action potential is shown, however, is 1.30 times that of the most irritable fiber in the parent trunk. The conduction rate of the fastest fiber in the first is 34.4 m.p.s. as against 25.3 for the fastest fiber in the latter.

The peroneal record which is added in order to have the action potential of a large mixed nerve for comparison has a relatively much greater first elevation than does either of the cutaneous nerve records. It is to be

TABLE 4

Ratio of conduction rates of elevations to that of the fastest elevation of the parent nerve

CUTANEOUS NERVE		ELEVATIONS			PARENT NERVE	ELEVATIONS		
		1	2	3		1	2	3
Post.	1–17	1.00	1.49	2.83	Tib.	1.00		
Post.	1–10	1.12	1.80	2.40	Tib.	1.00		
Med. 1	2–8	1.02	1.53		Tib. S.	1.00	1.51	2.42
Med. 2	2–8	1.00	1.52		Tib. S.	(same nerve)		
Plant	3–20	1.04	1.65		Tib. S.	1.00		
Averages.......		1.04	1.60	2.62				
Lat.	3–20	1.12	1.70	2.67	Per.	1.00	1.59	
Lat.	3–23	1.00	1.29	2.35	Per.	1.00	1.40	2.15
Dors.	3–1	1.28	1.66	2.21	Per.	1.00	1.59	
Dors.	3–14	1.22	1.71	2.00	Per.	1.00	1.64	
Dors. averages..		1.25	1.69	2.11				
Parent nerve averages..............................						1.00	1.55	2.29

TABLE 5

Ratios of the elevation heights (voltages) and areas of the second and first elevations

NERVE	HEIGHT	AREA
Posterior..	2.00	1.44
Dorsal..	2.31	6.29
Peroneal..	0.30	0.41

TABLE 6

	POSTERIOR 3-26	MEDIAL 3-4	PLANTAR 3-4	LATERAL 3-28	DORSAL 3-5
Diameter of largest fiber in microns...	19.0	17.5	18.1	15.0	12.8
Number of fibers above 12 microns.....	17	10	9	7	1

remembered in this connection that the peroneal nerve contains many fibers, both motor and sensory, which supply muscles. This, no doubt, accounts for the enormous first elevation present.

Correlation between reflex and action potential data. When one considers

together the two sets of data, that pertaining to the reflex thresholds, and that relating to the action potential elevation thresholds, it is at once suggested that the fibers which mediate the ipsilateral extensor reflex may be the ones which form the first elevation, while those which evoke the flexor reflex are responsible for the second. However, further experiments show that this is only partially true. The most irritable of the fibers mediating the extensor reflex are among those giving rise to the first part of the first elevation, but the range through which fibers that mediate this reflex extend is broader than that of fibers contributing to both of the action potential elevations. Isometric records of the reflex muscle contraction show that the muscle continues to develop tension through a range of fibers from those similar to the most irritable motor fibers to fibers whose thresholds are about three times as great. This means that the fibers which mediate this reflex extend at least through the first two elevations, and into the third. Stated in another, and perhaps better way, the fibers which mediate this reflex of the ipsilateral extensor are fibers which conduct at rates ranging from about 40 to 14 meters per second.

Similarly, those fibers which mediate the flexor reflex extend through a band of the fiber spectrum which is broader even than the range of fibers which mediate the extensor reflex. The most irritable of the fibers which mediate the flexor response have about the same irritability as those which contribute to the first part of the second action potential elevation, but as one increases the intensity of the stimulation, the tension developed by the muscle continues to show increments until a stimulus strength 8 to 10 times the intensity required to excite the largest motor fibers is reached. This means that the fibers extend deep into, or perhaps through the fourth or B elevation. The latter is consistent with the findings of Heinbecker, Bishop, and O'Leary (1933) that the fibers which mediate the sense of pain lie largely in the B range. This statement must not be taken to mean that the flexion reflex is a nociceptive reflex in its entirety. In fact, evidence is presented in another part of the study showing that in the near threshold range this is distinctly not the case. However, it very probably is true that the nociceptive fibers do contribute to the flexion reflex, though the range of fibers which evoke the flexion reflex is broader than that of fibers which carry pain impulses. To describe the fibers which evoke the flexor reflex in terms of conduction rate, one may say that they are fibers which conduct at rates of 27 to perhaps 6 or less m.p.s.

Morphological correlations. Osmic acid preparations of samples of all the different nerves studied were examined, and the diameters of the large fibers were measured. All of the myelinated fibers of one section of the posterior nerve were measured, but as only the fibers of the low threshold range can clearly be correlated with the other findings in this study, only the diameters of the large fibers will be given. Table 6 gives typical data

on the largest fibers from sections of selected examples of the various nerves.

All of the nerves show considerable morphological variations. The largest fiber, for example, in the posterior is sometimes as small as 17.5 μ, but has been found to be as large as 24. However, in no specimen of the dorsal has a fiber larger than 13.8 μ been found and the largest fiber may be as small as 10.8. Out of five sections of this nerve measured only one contained as many as two fibers as large as 12 μ, and two contained no fibers that large. In the sciatic, peroneal, and tibial nerves studied, the largest fibers lay within the same diameter range as that reported for the posterior, i.e., 17.5 to 24 μ. Therefore, the morphological findings appear to confirm completely the reflex and oscillographic data reported. The nerves which contain fibers of low threshold and rapid conduction, and which evoke the low threshold extensor response contain fibers within the size range of the largest fibers in the sciatic. The nerve which does not evoke the extensor reflex, or only to an almost imperceptible degree, but whose main reflex response is flexion, and which exhibits a higher threshold and slower conduction rate, does not contain such large fibers.

Central time relations. The finding that the optimally effective frequency of stimulation for the extensor reflex is in the region of 120 per second, while that for the flexor reflex is about 60 seemed to indicate that there is a fundamental difference in the time relations of the central processes for the two reflexes. In order to ascertain whether or not this is the case the central reflex time for each of the two reflexes was measured by recording them simultaneously upon a fast moving drum. The reduced reflex time for the ipsilateral extensor reflex was found to be 21.9 ms., while that for the flexor reflex is 34.4. In these measurements only those are considered for which stimuli of intensities of 1.36 times the flexor reflex threshold and greater were employed. It was found that when weaker stimuli were used there was great fluctuation in the latent period, especially that of the flexion reflex. One measurement of a record made by stimulation with shocks 1.05 times the flexion reflex threshold showed a gross flexion reflex latency of 260 ms. However with shocks of 1.36 times flexor reflex threshold and stronger, the reduced reflex time was fairly constant, with the flexion time still showing the greater variation.

At any rate, one unchanging result appeared in the records. Regardless of the strength of stimulation used and all other conditions, in every record of simultaneously recorded flexor and extensor reflex responses the extensor latent period was shorter than that of the flexor by at least 12 ms., and usually more. These figures on reduced reflex time are slightly longer than those of Buchanan (1908). She recorded the ipsilateral extensor (gastrocnemius) reflex of Rana temporia, and reported a reduced reflex time for the electrical response usually between 12 and 20 ms. This difference of 3 or 4 ms. may possibly be a species difference.

The two kinds of data on the central time relations of the two reflexes, namely, 1, that the optimally effective frequency for the extensor reflex is higher, and 2, that the latent period is shorter, indicate that central excitatory state builds up more quickly in the extensor center, but is of shorter duration or dissipates more rapidly. The extensor center exhibits the property of inertia, therefore, to a less marked degree than does the flexor center.

Discussion. The differences in speed of reaction of the flexor and extensor reflex arcs, peripherally and centrally, may conceivably be an instance of the difference between postural and locomotor mechanisms. It seems significant that Brondgeest (1860) found that in the frog, corresponding with the habitual posture of squatting, postural tonus is chiefly in the flexor muscles. J. Hay (1901) has reported that in the rabbit the motor fibers to red muscle average about 5 μ smaller than the motor fibers to pale muscle. It is known that the red muscles are slower in their contraction than are the pale muscles, and there is evidence which indicates that they are postural in function (D. Denny Brown, 1929). It appears a worth while speculation, therefore, that the ipsilateral extensor reflex in the bullfrog which is evoked, par excellence, by stimulation of the posterior nerve may be a locomotor reaction, while the non-nociceptive part of the flexor response may be postural.

Another phase of the problem which this study illuminated is the relative difficulty which other workers have experienced in evoking the ipsilateral extension reflex, and the variations in the quality of response they have obtained upon stimulation of large mixed nerves. Ranson (1931) and Ranson and Hinsey (1931) have doubtless inferred the real reason for the heterogeneity of responses. They attributed the mixed responses and difficulties of interpretation to the functional mixture of afferents excited.

It has been pointed out by Sherrington (1894) that the muscle afferent fibers supplying both the flexor and extensor muscles of the cat are large fibers, and there is no regularity of difference between them. If this is true in the frog, there are at least three functional groups of afferent fibers in the leg of the bullfrog which lie in the same threshold range. They are: first, proprioceptive fibers from the extensor muscles; second, similar fibers from the flexors; and third, large cutaneous fibers which evoke the extensor reflex. It is not surprising then that even in the low threshold range inconstant reflex responses are obtained when a large mixed nerve is stimulated. In the posterior and the other *cutaneous nerves containing large fibers*, however, there is apparently a quite broad band of fibers which are functionally homogeneous, thus accounting for the constancy of the response to their afferent stimulation.

All of the results of these experiments are consistent with the doctrine of "specific nervous energy." Excitation of a certain afferent fiber evokes only one type of muscular reflex response as shown by the muscles of the

hind limb. In one experiment, the frequency of stimulation of the posterior nerve was increased to more than 500 per second. The intensity was such that only low threshold fibers were excited. The triceps muscle responded by contraction. The semitendinosus remained quiet. All of the results in this study seem to indicate that the kind of reflex response evoked, and by inference the sensation experienced, when a nerve is stimulated depends upon the central distribution of arriving impulses, and not upon the frequency of arrival or other factors.

SUMMARY

The cutaneous afferent fibers which evoke ipsilateral extensor and flexor reflexes in the hind limb of the bullfrog have been identified and certain correlations derived.

The stimulation of different areas of the skin of the hind limb of the bullfrog evokes qualitatively different reflex responses in the muscles of that limb. Excitation of the fibers of the nerves supplying these different skin areas elicits corresponding reflex responses.

The optimally effective frequency of stimuli for the ipsilateral extensor reflex is found to be about 120 per second; that for the flexor reflex is about 60. The threshold of the extensor reflex at a frequency of 120 per second is similar to that of the most irritable motor fibers in the sciatic nerve. The threshold of the flexor reflex at its optimally effective rate is about 1.6 to 1.7 times this value.

The cathode ray oscillograph discloses that the cutaneous nerves which primarily evoke the ipsilateral extension reflex contain fibers which are as irritable as the lowest threshold fibers in the sciatic nerve, and whose rates of conduction are similar to those of the most rapidly conducting fibers of the sciatic. The nerve which does not evoke the extensor reflex, or to only a very small degree, but whose main response is flexion does not contain fibers with such low thresholds and rapid rates of conduction.

Morphologically, the nerves which best evoke the extensor reflex are found to contain fibers as large as the largest fibers in the sciatic, while the largest fibers in the nerve which evokes the flexor reflex as practically its only muscle response average 6 to 10 μ smaller in diameter.

The reduced reflex time for the extensor reflex is found to be shorter than for the flexor reflex. This finding considered together with the higher optimally effective frequency of stimulation and the fact that the afferent fibers are larger indicate that both the central and afferent peripheral processes are faster for the extensor reflex than for the flexor.

These differences may signify that they are postural and locomotor mechanisms.

The flexion reflex elicited by excitation of cutaneous nerve fibers, in the light of certain findings in this study, cannot be considered as entirely nociceptive in origin.

In the fiber "spectrum" the band occupied by the extensor reflex afferent fibers ranges from fibers which conduct at rates from about 40 to 14 m.p.s.; that occupied by the flexor fibers from about 27 to 6. The ranges, therefore, are wide and overlap. When excited by distinctive strengths of stimuli an increase in rate is without effect on the quality of the responses. This may be considered as evidence supporting the doctrine of "specific nerve energy."

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THE EFFECT OF TOTAL THYROIDECTOMY UPON EXPERIMENTAL DIABETES INSIPIDUS IN DOGS

WILLIAM MAHONEY AND DONAL SHEEHAN[1]

From the Laboratory of Physiology, Yale University School of Medicine

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While investigating the experimental production of diabetes insipidus, the problem of correcting the upset water balance presented itself for study. Reasons for believing that the thyroid is implicated are to be found in recent literature and the present communication gives a brief account of experiments bearing upon this phase of the problem.

In 1910 Crowe, Cushing and Homans (1) showed in dogs that transection of the pituitary stalk resulted in a transient severe polyuria, and shortly thereafter Cushing and Goetsch (2) reproduced the phenomenon by placing a "silver clip" upon the pituitary stalk. Although polyuria has been noted after hypophysectomy, and following lesions of the hypothalamus, more consistent and lasting effects have been produced by damage to the stalk. We have depended upon this last method for the present study. The duration of the polyuria has been variable, so for this investigation only animals which showed polyuria for at least two weeks were selected for subsequent thyroidectomy.

The importance of endocrine interrelationships has been recognized for some time and it has been felt more recently, as facts have been unfolded, that the controlling mechanism is to be sought in the pituitary body. One of the first connections noted was that between the pituitary and thyroid. In 1889 Rogowitsch (3) described enlargement of the pituitary body following thyroidectomy. This was confirmed by Herring (4) in 1908, who noted in particular an "increased activity" of the pars intermedia with a great increase in the number of hyaline bodies in the pars nervosa. Crowe, Cushing and Homans (1) in 1910 observed the reversal, viz., a hyperplasia of the thyroid in the first 48 hours following hypophysectomy, but later functional involution of the thyroid with excessive accumulation of colloid in the vesicles.

In 1920 Strauss (5) reported the case of a boy who developed diabetes insipidus at the age of nine years. When the patient was thirteen years old the polyuria and excessive thirst progressively disappeared with the gradual onset of myxedema. At fifteen years of age the boy was treated by thyroid gland administration with amelioration of the myxedematous

[1] Rockefeller Fellow.

state, but no mention was made concerning the subsequent water balance. The patient died of pneumonia in his twentieth year. No post-mortem examination was made. Barnes, Regan and Bueno (6) in 1933 noted that the marked diuresis which occurred in normal animals following the injection of extracts from anterior bovine pituitaries, was not observed in thyroidectomized dogs. They suggested that the diuresis was the result of thyroid activity. Their observation has been confirmed by Biasotti (7).

METHOD. Healthy mongrel mature and immature dogs were the subjects for study. They lived in the laboratory in large metabolism cages for two to four weeks before any operative procedure was undertaken. Daily measurements were made of the total fluid intake and urinary output. No direct estimations of fluids lost by way of intestines, skin and lungs were made, but the "insensible" loss was calculated by computing the difference between the fluid intake and urinary output. Diets were kept constant throughout the experiment.

The operative procedures were carried out under sodium amytal anesthesia. A median scalp incision was made; the right zygoma was resected; the right temporal muscle was incised and reflected downward as a flap, later to be used in the closure; the bone over the right parietal and temporal regions was removed, and the dura opened widely. The right temporal lobe was elevated and the pituitary body exposed, so that a silver clip was applied to and closed about the pituitary stalk under direct vision. There was usually no bleeding, and closure was done by resuturing the divided temporal muscle and scalp. Within twelve hours the animals recovered sufficiently from amytal anesthesia to begin drinking. During the preceding period of unconsciousness they passed considerable quantities of urine.

Thyroidectomies were performed through a midline skin incision and care was taken to leave the parathyroid glands intact. The excised tissue was in all instances verified histologically. On no occasion was there any manifestation of tetany. The dogs were surprisingly active after thyroidectomy, and showed no gross signs of thyroid privation. There was, however, a remarkable and steady loss of weight after removal of the thyroid. It was found that, after prolonged thyroid feeding, diarrhea frequently followed. In the observations presented thyroid feeding was discontinued immediately upon the appearance of diarrhea.

OBSERVATIONS. Following clipping of the pituitary stalk in dogs, an immediate elevation of the fluid intake and urinary output invariably occurred. The daily measurements reached about ten times the preoperative readings, but frequently fell after forty-eight hours to five or six times the original quantities. The condition of polydipsia and polyuria then persisted at this level for several weeks and, in some cases, for a period of two months.

In some experiments the postoperative fluid intake was limited to the

average quantity ingested daily before operation. Under these conditions polyuria was not manifest, although the animal showed all signs of excessive thirst. The polyuria appeared immediately the limitation of fluid intake was removed.

The following protocol illustrates the striking effect of thyroidectomy and subsequent thyroid feeding in an adult dog in which the water balance had been disturbed previously by occlusion of the pituitary stalk. Figure 1 shows graphically the details of this experiment.

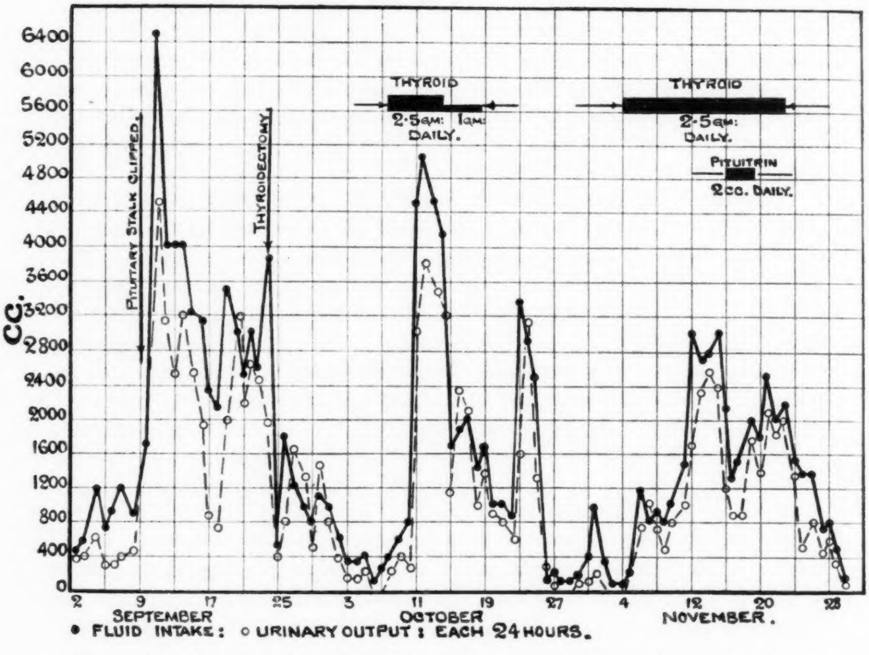

Fig. 1. To show the effect of thyroidectomy and subsequent thyroid feeding on the upset water balance of an adult dog, weight 14.9 kgm., in which the pituitary stalk had been previously occluded.

An adult mongrel bitch weighing 14.9 kgm. before operation had a daily fluid exchange averaging 800 cc. intake and 400 cc. output. Immediately following occlusion of the pituitary stalk the fluid intake rose to 6500 cc. and the output to 4500 cc. per day. After three days the fluid exchange dropped somewhat but remained at a high level, fluctuating between 2500 and 3800 cc. per day. Fifteen days after occlusion of the pituitary stalk total thyroidectomy was performed. Within twenty-four hours the intake had dropped from 3800 cc. to 500 cc. and the output from 2000 cc. to 600 cc. There then followed a gradual fall of the fluid exchange almost to the base line so that the animal was ingesting not more than 200 cc. of fluid daily and the urinary output was so scant as scarcely to be measurable. Replacement therapy

with desiccated whole thyroid gland, 2.5 grams daily by mouth, was begun. Within three days the daily fluid intake remounted from 400 cc. to 5000 cc. and the output from the base line to 3800 cc. It maintained this high level until the thyroid administration was lowered to 1 gram daily, following which the intake dropped to 1700 cc. and the output to 1200 cc. After eleven days of thyroid therapy all glandular administration was stopped, and apart from a spontaneous and unexplainable three day elevation in fluid exchange which occurred exactly simultaneously in other animals in the same laboratory, there was a gradual and steady fall of the fluid exchange to the base line. This low level was maintained for nine days when thyroid gland administration (2.5 gm. per day) was begun again. The rise of fluid exchange at this point, instead of being abrupt was more gradual and the fluid intake ultimately reached a height of 3000 cc. and the output 2600 cc. The thyroid therapy was continued, and, in addition, pituitrin (obstetrical) 1 cc. was given subcutaneously twice daily over a period of three days; during this time the intake dropped to 1300 cc. and the output to 900 cc. After withdrawal of the pituitrin, while the thyroid administration continued, the fluid exchange remounted to 2500 cc. All thyroid feeding was discontinued and the fluid exchange once again fell to a base line level. The animal is still alive and has lost 5 kgm. in weight ($\frac{1}{3}$ of its initial body weight) since thyroidectomy.

The sharp fall in the fluid intake and output following thyroidectomy and the subsequent reappearance of polyuria and polydipsia during thyroid gland administration, illustrated in the above protocol, has occurred without exception in five animals in which the above procedures were carried out after occlusion of the pituitary stalk.

When thyroidectomy was performed simultaneously with occlusion of the pituitary stalk there followed moderate polyuria and polydipsia for a brief period of twenty-four to forty-eight hours, and these states were effectively reproduced by subsequent thyroid administration.

It became obvious that studies of the water balance were required after thyroidectomy alone without previous occlusion of the pituitary stalk, as well as of the effect of thyroid gland administration to normal dogs. Figure 2 illustrates that thyroidectomy is followed by no demonstrable effect upon the urinary output of the normal dog. The intake was exaggerated more than the output after thyroidectomy. The insensible loss of this animal must therefore have been considerable since this subject (a puppy aged 5 months) gained no weight over a period of three months. The difference in the response of water metabolism to thyroid feeding before and after occlusion of the pituitary stalk is strikingly demonstrated in this experiment. Figure 3 is presented as an example of several studies upon dogs *without operation* to show the influence of thyroid feeding on fluid exchange. It will be seen that the daily fluid intake and urinary output rose gradually to almost twice the original levels. The dosage of thyroid therapy given was 2.5 grams daily, admittedly a considerable quantity; but this was done in order that the animals might serve as fair controls for the experiments which involved thyroid administration after occlusion of the pituitary stalk.

In addition to polydipsia and polyuria the application of a silver clip to

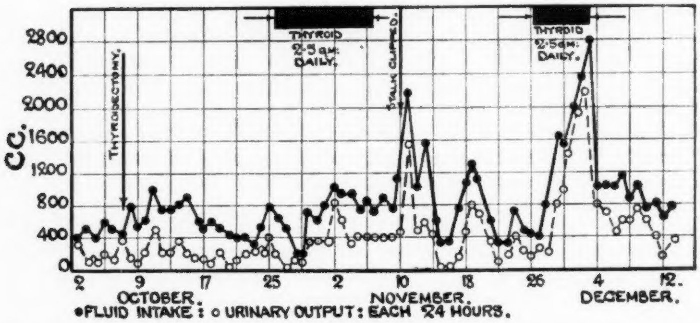

Fig. 2. To show lack of any demonstrable effect of total thyroidectomy upon the water balance of the normal dog. Subsequent occlusion of the pituitary stalk produced a polyuria and polydipsia for one day only. This condition of upset water balance was reproduced by thyroid feeding.

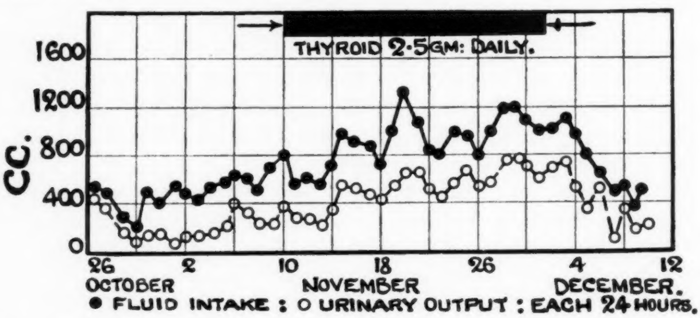

Fig. 3. To show the slight diuresis produced in the normal dog following thyroid administration.

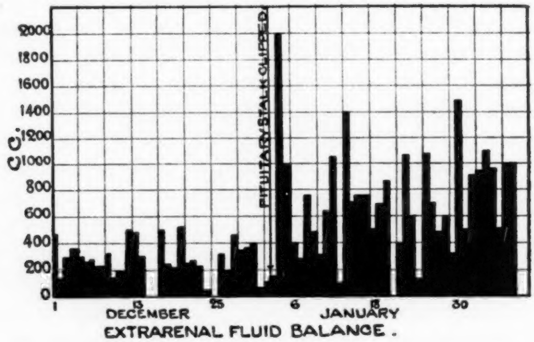

Fig. 4. To show the effect of occlusion of the pituitary stalk upon extrarenal water balance of the dog. The black columns indicate the daily difference between fluid intake and urinary output.

the pituitary stalk was frequently followed by a marked upset in the fluids lost by other routes (intestines, skin and lungs). This took the form of an exaggeration of the excess of fluid intake over urinary output with wide variations in both readings from day to day. Thyroid administration appeared to aggravate this condition. The results indicate a severe imbalance in water metabolism apart from renal excretion. The effect of thyroid on the fluid exchange in tissues might be explained by a rapid breakdown of metabolites with the consequent demand for fluid, but a consideration of the omnipresent dilute urine (specific gravity 1.002 to 1.005) during the periods of polyuria indicates that more fluid had been supplied than should have been necessary to maintain osmotic equilibrium.

SUMMARY

1. In dogs, occlusion of the pituitary stalk with a silver clip is followed by extreme polyuria and polydipsia.

2. This effect is abolished by subsequent total thyroidectomy, and reëstablished by oral administration of desiccated whole thyroid gland. Alternate states of extreme polyuria and oliguria could be produced at will.

3. Total thyroidectomy has no demonstrable effect upon the daily fluid intake and output of the normal dog.

4. In dogs with intact pituitary stalk, thyroid feeding has a mild diuretic action. This effect is, however, in no way comparable to the extreme polyuria and polydipsia produced by administration of thyroid gland after occlusion of the pituitary stalk.

5. A severe imbalance of the water metabolism of the tissues apart from the renal mechanism is evident after occlusion of the pituitary stalk in dogs.

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THE EFFECTS OF MAGNESIUM DEFICIENCY ON THE TEETH AND THEIR SUPPORTING STRUCTURES IN RATS[1]

HENRY KLEIN, ELSA R. ORENT AND E. V. McCOLLUM

From the Department of Biochemistry, School of Hygiene and Public Health, Johns Hopkins University, Baltimore, Maryland

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When young rats and dogs are fed a ration containing only 1.8 parts per million of the element magnesium, but adequate amounts of other known dietary essentials, they develop a series of symptoms which indicate that in these species, magnesium is essential for life (1), (2), (3), (4), (5), (6), (7).

Such a dietary regimen produces marked pathological lesions in the mouths. The results on rats are herein described.

Experimental. Mouth examinations were made on twenty-four rats which when weaned were placed on the low magnesium diet (2). They were killed or died at various ages as indicated in table 1. The heads were removed and prepared for histological examination using Zenker fixation, eosin staining and hematoxylin. Paraffine imbedding was used for young animals and celloidin for older individuals.

Results. In the control rats, the appearance of the mouth is that shown in figure I. The mouths of rats fed the low magnesium diet present marked differences (fig. II). These appear gradually, as noted in the table, become marked after approximately one month on the diet, and extreme after three months.

Inspection of the maxilla (fig. II), reveals the extent of the change in the tissues. The mucous membranes appear blanched. The gingival tissue, which usually is closely adherent to the bone and the molar and incisor teeth at their gingival margins, is, in the rats fed the low magnesium diet, in both upper and lower jaws, a bulbous mass of smooth whitish grey tissue. Particularly in the lower jaw, the incisor teeth do not lie in close proximity to each other as is usually the case in normal animals but are widely separated at their proximal surfaces by the tissue mass. In the molar regions, darkly colored, brown and yellow, irregularly shaped hard ma-

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terials are imbedded in the tissue mass and protrude above it. The position of these particles of material is that usually occupied by the molar teeth. No structure which suggests the usual molar tooth morphology, is apparent on inspection.

When a small dental probe is applied to the irregularly shaped hard structures imbedded in the tissue masses, these particles become dislodged. When removal of these particles is accomplished, the molar teeth are seen lying deep within the tissue masses. The removed structures appear to be deposits of calculi. The molar teeth after such exposure

TABLE 1

RAT	DAYS ON DIET	GINGIVAL TISSUE SWELLING	MACROSCOPIC DENTAL DECAY
1097	3	None	None
1099	6	None	None
1101	11	None	None
1102	12	Slight	None
A28	12	None	None
A27	12	Very slight	None
A29	14	None	None
A30	14	None	None
A31	15	Very slight	None
A32	17	Very slight	None
A33	17	Slight	None
1106	17	Slight	None
A34	19	Slight	None
A35	22	Slight	None
A36	24	Slight	None
A37	24	Slight	None
A42	36	Marked	None
A43	38	Marked	None
A44	39	Marked	None
A26	52	Marked	None
A25	76	Marked	None
A41	83	Extreme	None
A38	99	Very marked	None
A40	106	Extreme	None

appear relatively unabraded and free from evidences of macroscopic decay. They lie loosely in the tissue mass and may be readily lifted out with forceps.

The incisor teeth appear somewhat longer than in the controls and their labial surfaces are pitted and rough, especially at the gum margin.

Microscopic examination: Controls. The teeth of rats fed the control diet (low magnesium diet plus added magnesium) show on histological section the usual arrangement and structures. As shown in figure III, the three molar teeth lie close together in the antero-posterior position. An-

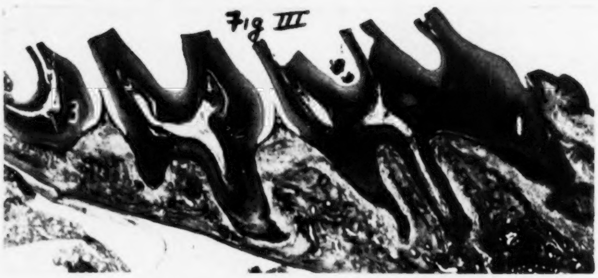

Fig. I. Maxilla of rat on control diet; age 127 days. *I*, incisors; *1*, 1st molar; *2*, 2nd molar; *3*, 3rd molar.

Fig. II. Maxilla of rat on low magnesium diet; age 127 days.

Fig. III. Arrangement of molar teeth of rat fed control diet. Control for rat A 38 magnification ×15. *1*, 1st molar; *2*, 2nd molar; *3*, 3rd molar.

Fig. IV. Arrangement of molar teeth of rat fed low magnesium diet. Rat A 38 magnification ×10.

Fig. V. Section bone and paradontium control rat 17 days on control diet after weaning. Magnification ×120. *D*, dentine; *P*, paradontium; *B*, bone.

Fig. VI. Section bone and paradontium low magnesium rat on diet 17 days after weaning. Magnification ×120. *D*, dentine; *P*, paradontium; *B*, bone.

teriorly the highest tip of the gingiva lies well below the highest point on the anterior cusp of the first molar. The junction of the tooth epithelium and the gingival epithelium is clearly discernible. The epithelium covering the gingival crest at this point is relatively thin. Beneath this epithelium lies well organized connective tissue. The nuclei are abundant and narrow. These same characteristics are apparent in the gingival crests which lie between the molars. A relatively thin paradontium covers the roots of all the molars. The nuclei of the connective tissue cells of the paradontium are long, narrow and abundant. On the mesial side of the first molar, alveolar bone extends up close to the base of the gingival crest, completely borders the roots against the narrow paradontium, fills the space between the molar roots relatively completely and extends well up to the bases of the gingival crests between all the molars. The bone stains deeply with eosin and only in few areas are there evidences of osteoclastic activity.

The dentine of the molars is uniform in structure, taking a dense hematoxylin stain. The pulp presents the usual characteristics. The molar cusps are worn. No dental decay is apparent. Those portions of the incisors which lie within the jaw (root) present the usual appearance: dentine stained uniformally, the ameloblastic cells and layer unbroken, the subameloblastic layers usual in appearance and below these a layer of dense bone.

Low magnesium rats. With the histological technique used no apparent differences in the paradontium, bone or tooth structures are noted in rats fed the magnesium deficient diet for 3 or 6 days. By the 11th day, however, changes become evident although they are exceedingly fine. Study of the sections indicates that the small observed changes are definite. The nuclei of the cells of the paradontium are more irregular in size and more stellate in shape. The bone bordering the paradontium shows an excessive blue-staining amorphous border over and above in amount that appearing in the controls. No changes are apparent in the developing portions of the incisors at this stage.

After feeding the diet 17 days, the changes, although still of fine character, are more definite and of the same character as described for the earlier stage. The most predominant feature is a change in the character of the cells and intercellular substance of the paradontium. The cells appear larger and are separated from each other by large amounts of pink staining intercellular material. In the controls the cells are long and narrow and closely arranged. These differences may be noted in figures V and VI.

Examination of sections from rats fed the low magnesium diet for three months shows marked differences in the character of the supporting structures of the teeth as well as changes in the teeth themselves. The molar

teeth (fig. IV) lie imbedded in a mass of tissue which stains readily with eosin. Within this mass of tissue lie many spindle shaped cells having large deep blue-staining round or oval nuclei. The usual character of these portions of the gingiva which lie between the molars is changed. The height of the gingival crests lies above the highest point on the molar teeth. The subepithelial connective tissue is increased in amount and is made up of many spindle shaped faintly blue-staining cells having large round or oval nuclei and imbedded in more than usual amounts of faint pink-staining ground substances. The epithelium covering this tissue mass is thickened and shows many extensions into the underlying tissue. Alveolar crests of bone are absent from the usual position about the roots of the molars. The paradontium is not recognized as such for it is intimately fused and appears identical in structure with the bulbous tissue mass. The bone present contains an abundance of marrow spaces and large amounts of an amorphous material taking a deep blue stain. This material chiefly borders the edges of the bone.

The cusps of the molars appear relatively unabraded and are covered in part by heavy deposits of a deep blue-staining substance having some of the characteristics of dental calculus. The dentine of the molars is dense but contains many striations suggesting an intermittent disturbance in its calcification. The pulp tissue is dense and contains very large numbers of closely packed cells, some stellate in shape. The portions of the incisor teeth which lie within the jaw show changes from the controls. The pulp contains large amounts of a deep blue-staining substance, dense and amorphous in structure and continuous with a layer of well organized dentine. Striations in the dentine similar to those in the molars are present. The ameloblastic layer also shows changes. The position of this layer is occupied by a thin deeply blue--staining ribbon-like structure. The layers immediately adjacent labially, usually occupied by the cells of the papillary layer and the stratum intermedium, are filled with a dense deep pink-staining tissue containing many spindle shaped cells with large round or oval nuclei. The connective tissue which lines the dentine on the lingual side of the incisor root, is filled with a thick mass of tissue the characteristics of which are the same as those already described for the tissue surrounding the molar teeth.

Discussion. The question of the necessity and identity of various and specific food substances for physical well-being has occupied the attention of investigators for some time. That many food substances are necessary for the development of normal teeth is, in addition, well recognized. In this connection may be mentioned the significance of adequate and proper levels of calcium and phosphorus, vitamins A, C, D and possibly the B complex. The importance of other inorganic and organic substances when restricted or fed in excess in the diet, in relation to structural excellency of teeth, is as yet to be investigated.

In an attempt to further develop this special field, the effects of strontium inclusion in the diet have already been noted (8). To these data may be added the effects herein described, indicating that the element, magnesium, when restricted in the diet, produces massive pathological changes in the teeth and their supporting structures.

The findings indicate that magnesium restriction in the diet produces its most marked effects upon the character and growth of the cells and intercellular substance of the paradontium. This massive effect upon the membrane which lines the roots of the teeth and adjacent alveolar bone, appears specific with this particular food deficiency. In studies on more than 1500 heads of rats fed a variety of defective diets, none has shown a condition of like character.

The mechanism by which magnesium deficiency induces the described changes in the paradontium is not entirely clear. The studies indicate, however, that changes in the relation of the number of cells to the concentration of intercellular substance appear early and probably first. Undoubtedly the paradontal changes predominate after longer feedings of the deficient diet. These findings, when considered with the chemical studies on this deficiency (5) (6) (7), suggest a possible explanation for the origin of the described pathological changes. The presence of deep blue-staining amorphous material lining the borders of the alveolar bone, especially in the young experimental animals, may be interpreted as a result of precipitation of calcium salts. Although such blue material is present in the sections of control animals, the extent and amounts are less in the latter. It is possible, of course, that the differences in amounts of this substance may be accounted for in part by differences in degree of decalcification in preparation for sectioning. The published chemical findings, however, indicate that favorable conditions are present for precipitation of calcium salts, for there is an increase in the absolute amounts of calcium and, to a lesser extent, of phosphorus in the long bones of the animals.

Coincident with these chemical changes which result in increased weight of the bones, the paradontal changes proceed (note table 1), so that a definite although slight swelling of the tissues about the teeth is present after 15 days. The nature of these chemical findings (calcium and phosphorus retention) makes it improbable that the increase in amount of abnormal paradontal tissue is primarily the result of bone resorption followed by a compensatory increase in the paradontal tissues in an attempt to hold the teeth in place in the mouth. If would appear, therefore, that the effects on the paradontal tissues arise largely from other factors. The picture presented by the sections of the older rats (absence of bone around the teeth and the presence of excessive amounts of abnormal connective tissue) probably results because the tissue proliferation proceeds so rapidly and is of such magnitude as to present the possibility that the teeth are

moved out and away from the alveolar bone by the massive increase in the paradontal tissues.

The chemical findings, although indicating the retention of calcium and phosphorus during early growth, show that longer feeding of the magnesium deficient diet results in excessive loss of calcium and phosphorus through the urine and feces. The character of the alveolar bone in older animals seems to reflect these changes (tendency to highly cancellous character) and may at this later stage in the development of the magnesium deficiency account for part of the excessive paradontal proliferation as an attempt by connective tissue to compensate for decreased density of bone.

The majority of the animals restricted to the deficient diet die early in life. A few survived the spectacular seizures already described (2). These are the animals which show the interesting striations in the dentine which, perhaps, reflect the intermittent character of the seizures and the following recoveries.

SUMMARY

The feeding of a diet low in magnesium is associated with decreased cell content and increased amounts of pink-staining intercellular substance in the paradontium, and also with the formation of a deep blue-staining amorphous material in the bone lining the paradontium after 17 days of feeding.

Sections of jaws of animals fed for three months show absence of bone around the molar teeth and positional substitution by large deep pink-staining masses of tissue containing many spindle shaped cells.

The incisor teeth are surrounded by a similar mass and the tooth structures themselves show marked change, especially the persistently growing incisor roots.

These changes indicate that magnesium is essential for proper formation of the teeth and their supporting structures in the rat.

The possible mechanisms by which these changes arise are discussed.

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GROWTH AND GLYCOGEN CONTENT OF THE FETAL LIVER AND PLACENTA

E. L. COREY

From the Physiological Laboratory of the University of Virginia Medical School[1]

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The work of earlier investigators has led to a general understanding of carbohydrate metabolism in the developing embryo and fetus, particularly in regard to the placenta and fetal liver (Needham, 1931). Authors appear to agree on the early glycogenic function of the placenta, first brought to the attention of physiologists by Claude Bernard in 1859. It is indicated furthermore that the initiation of glycogenic activity in the fetal liver occurs when the glycogen concentration in that organ first exceeds that of the placenta.

Demant (1887) was able to detect the presence of glycogen in the livers of term puppies in a concentration as high as 11 per cent. Positive tests were obtained for this substance from the embryos of the cow, sheep and pig by Pflüger (1903). Mendel and Leavenworth (1907) found no trace of glycogen in pig fetuses of 85 to 230 mm. in length, although it was present during the later stages of gestation.

In a study on the fetal rabbit, Lochhead and Cramer (1907) observed that the placental glycogen remained fairly constant until the 24th day of gestation, after which it fell steadily until term. The fetal liver glycogen, on the other hand, increased up to the 25th day of development to a value above that found in the placenta. These investigators concluded that "this . . . represents the date at which the liver assumes its adult glycogenic function." Snyder and Hoskins (1928) also used the rabbit and reported that the glycogen content of whole fetuses rose during the latter third of gestation from a trace to a percentage higher than that in the maternal liver. Little appears to have been added to our knowledge of the subject, therefore, within almost thirty years past.

Methods. Pregnant rats of Wistar Institute[2] strain, maintained on a standard diet and fasted for 12 hours, were stunned sufficiently to stop muscular movements but without arresting respiration or cardiac activity.

[1] Acknowledgment is made of aid received in the above investigation from the Committee for Research in Problems of Sex of the National Research Council.

[2] The rats used in this investigation were of pure Wistar Institute strain, the original stock being obtained through the kindness of Dr. M. J. Greenman.

Immediate laparotomy and rapid excision of maternal and fetal tissue samples were then carried out. Determinations were made of *a*, solid, and *b*, glycogen content of fetal livers by pooling tissues from several fetuses of one litter in each instance; commonly 4 fetuses were used for *a* and 3 for *b* analyses. All fetuses were alive and apparently in good condition at the time of isolation of the liver tissues.

Whole placentae were used for wet weight and total solid data as well as for glycogen determinations, since it was not feasible to separate fetal and maternal portions of that organ. Analyses for glycogen were made according to the modification of Pflüger's method employed by Silvette and Britton (1932) for amounts of tissue from 0.5 to 1.0 gram. Over 200 fetuses from 30 litters were used in the present study.

Growth of the fetal liver. Figure 1 presents the curve obtained when fetal liver weight was plotted against that of the entire fetus. It was apparent that the liver developed at a rather regular rate when the animals were between 1 and 5 grams in body weight. Determinations of the solid constituents of the liver indicated that an increasing degree of liver hydration occurred as gestation proceeded.

The relation of hepatic weight to that of the fetus is shown in figure 2. It is readily apparent that liver growth practically keeps pace with that of the fetus as a whole during the greater part of the gestation period observed, although a rapid decline in liver weight relative to that of the fetus is indicated during earlier fetal development.

Growth of the placenta. The rate of placental growth was found to be rapid (fig. 3) until about the 3-gram stage of fetal body weight; thereafter there was little further increase in placental weight. Total solid determinations on the rat placenta indicated an increase in placental hydration during the period of rapid growth, as shown in figure 3.

The growth of the rat fetus and that of the placenta were found to be quite dissimilar. The placenta reached approximately its maximum development at the 3-gram stage of gestation, in contrast to the progressive increase in fetal weight until parturition.

Glycogen content of the fetal liver. The fetal liver glycogen increased from approximately 1 per cent in the youngest fetuses examined (about 0.4 gram) to over 6 per cent at term. Figure 4 presents the data graphically. The rise in the fetal liver glycogen was found to be fairly rapid throughout the portion of the gestation period studied. Beyond the 2-gram stage, the fetal liver glycogen concentration was observed to exceed the average maternal level, and at term the fetal value was over twice that found in the mother.

Placental glycogen. In the case of the youngest fetuses examined (about 0.3 gram) the placental glycogen was found to be 1.21 gram per cent. No fetal liver glycogen determinations were possible at this early stage. In

0.4-gram fetuses, however, the liver glycogen was observed to be 1.10 per cent, while the placental level was 1.07 per cent. From these values it was apparent (see fig. 4) that the glycogen concentration of the fetal liver first exceeded that of the placenta at some time between the 0.3- and 0.4-gram stages. It seems probable that this "cross-over" point represents the beginning of adult hepatic function, as indicated above.

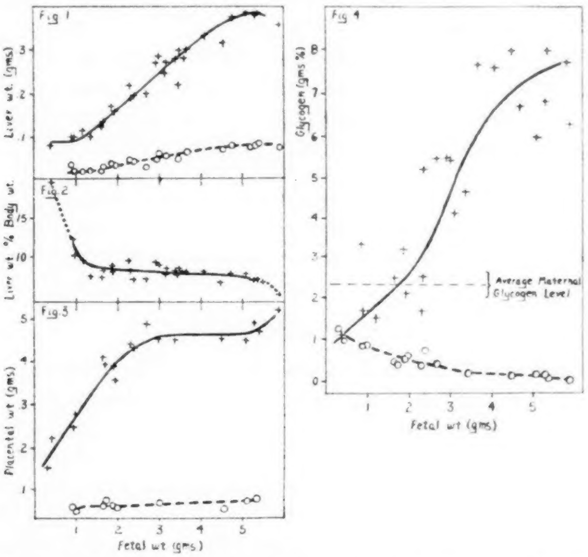

Fig. 1. Growth of the fetal liver showing wet weight (+, solid line) and total solid (○, broken line) determinations.

Fig. 2. Curve showing the percentage of fetal body weight represented by the liver.

Fig. 3. Graphic representation of placental growth; + (solid line) = wet weight; ○ (broken line) = total solid.

Fig. 4. Glycogen content of the fetal liver and placenta; + (solid line) = fetal liver glycogen; ○ (broken line) = placental glycogen.

The placental glycogen thereafter fell steadily until term, when it was found to be 0.05 gram per cent. If the "cross-over" point be expressed in percentage of the entire period of gestation, it may be calculated, by employing the data of Angulo (1932), that the assumption of adult glycogenic function by the fetal liver of the rat occurs after the elapse of 75 per cent of the total gestation period. It is of interest to compare this figure with the calculations of Needham (vol. ii, p. 1024), who found the corresponding point to occur at the end of 82 and 91 per cent of the total developmental period in the chick and the rabbit respectively.

It is the contention of Huggett (1928) that, in the rabbit, the maternal portion of the placenta acts in the nature of a "reserve" supply of glycogen for the developing fetus. In his experiments, it was observed that reduction of the maternal liver glycogen, or its enhancement by carbohydrate feeding, produced no effect on the placental glycogen content. Furthermore, Britton (1930) found that the fetal blood sugar level (cat) might remain within normal limits even during insulin-induced hypoglycemic convulsions in the mother.

Observations made on maternal and placental glycogenic levels in the course of the present investigation are in agreement with the above contentions, the maternal liver glycogen varying within wide limits (1.63 . . . 5.3 grams per cent) although the placental glycogen values remained relatively constant.

The present study was undertaken with the view of establishing normal glycogen values to serve as a basis for further observations on materno-fetal carbohydrate metabolism as related particularly to cortico-adrenal function.

SUMMARY

The rate of growth of the liver and placenta of the fetal rat during the latter third of the gestation period has been determined.

A progressive hydration of the fetal liver was evident throughout the period of development studied, the placental water content remaining, however, relatively constant from about the 3-gram stage until term.

The rate of growth of the fetus as a whole exceeds that of the liver until a body weight of slightly over 1 gram is attained, after which general bodily growth and that of the liver progress at relatively the same rate.

The placenta of the rat was found to increase rapidly in size until the fetus attained a body weight of about 3 grams, after which no readily demonstrable change occurred.

At a fetal weight of approximately 0.3 gram, the fetal liver glycogen concentration exceeded that of the placenta. This point of development probably represents the beginning of adult glycogenic function in the liver of the rat.

The placental glycogen concentration fell at a relatively constant rate from the earliest stages studied until birth, although wide variations were recorded in the maternal liver glycogen. This observation supports the contention that the placental glycogen content is relatively unaffected by varying carbohydrate concentrations in the mother.

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A COMPARATIVE STUDY OF SYMPATHIN AND ADRENINE

W. B. CANNON AND A. ROSENBLUETH

From the Laboratories of Physiology in the Harvard Medical School

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In previous communications from this Laboratory it has been shown that sympathin, i.e., the sympathomimetic substance which diffuses into the blood stream when sympathetic nerves supplying autonomic effectors are stimulated, differs from adrenine (Cannon and Rosenblueth, 1933; Rosenblueth and Morison, 1934; Cannon and Rosenblueth, 1935) and that sympathin from different sources may have different properties (Cannon and Rosenblueth, 1933). These facts led us (1933) to postulate the existence of two sympathins, one possessing exclusively excitatory effects (sympathin E), the other, exclusively inhibitory action (sympathin I).

This view has been contested by Bacq (1934) who points out what he considers weak aspects in our theory and offers alternative hypotheses to account for the data. We believe that a comparative survey of the action of adrenine and of sympathins from several sources on several indicators should aid in clarifying these conflicting suggestions. Such is the purpose of the present study. We shall first present what may appear to be heterogeneous experimental results, but they will find a natural place in the tables in which we shall summarize the data that are pertinent to a general discussion of the problem.

Results. *The influence of sympathin on the blood pressure.* Stimulation of the cardio-accelerator nerves (c.a.) in cats under dial anesthesia, or spinal and curarized, leads to slight or no changes of blood pressure, attributable to cardiac acceleration (fig. 1A). These changes subside shortly after cessation of the stimulus. If cocaine (8 mgm. per kgm.) is injected intravenously an initial rise of blood pressure, induced by stimulation of the c.a., is succeeded by a delayed and prolonged further rise (fig. 2A). This delayed response is attributable to cardiac sympathin, for its duration and maximal effects outlast the stimulus period and its time relations are closely parallel to those of the contraction of the denervated nictitating membrane used as an indicator of sympathin.

After ergotoxine (2 to 4 mgm. per kilogram), as shown by Dale (1906), adrenine causes a fall of blood pressure. C.a. stimulation may likewise induce a fall (fig. 1B), explainable as due to a decreased output of the heart in consequence of the acceleration. After ergotoxine and cocaine, adrenine

produces a fall, while cardiac sympathin elicits a delayed rise of blood pressure (fig. 1C). Thus cardiac sympathin is similar to hepatic sympathin (Cannon and Rosenblueth, 1933) and differs from adrenine.

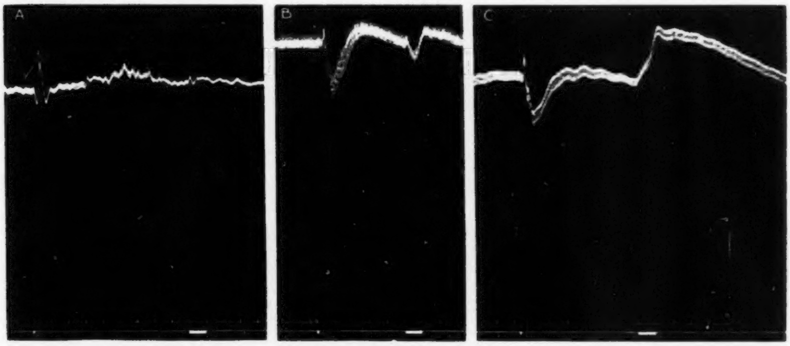


Fig. 1. Dial. Vagi cut. Effects of injecting 0.5 cc. adrenine (1:100,000) and of stimulating the right cardio-accelerator nerves (coil distance, 12 cm.). The heart rate increased in all instances. In this and the succeeding figures the time signal records 30-second intervals.

A. Before ergotoxine and cocaine. B. After ergotoxine (4 mgm. per kgm.). C. After cocaine (7 mgm. per kgm.).

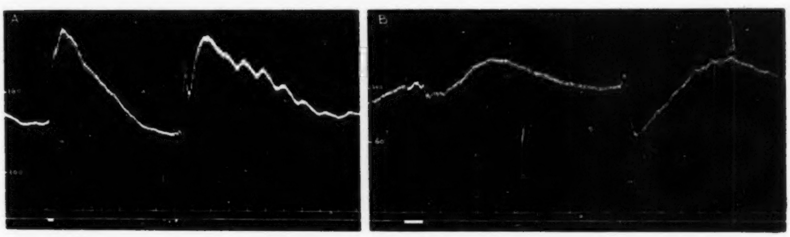


Fig. 2. Dial. Vagi cut. Cocaine (8 mgm. per kgm.). Effects of stimulating the right cardio-accelerator nerves and of injecting 0.3 cc. adrenine (1:100,000).

A. Before yohimbine. Cardio-accelerators stimulated for 10 seconds; coil distance, 10 cm. B. After yohimbine (3 mgm. per kgm.). Cardio-accelerators stimulated for 30 seconds; coil distance, 8 cm.

Yohimbine (3 mgm. per kilogram) is also capable of differentiating sympathin from adrenine. After yohimbine adrenine yields a fall of blood pressure (Hamet, 1925), while sympathin induces a rise, as illustrated in figures 2B, and 3. Injection of both yohimbine and ergotoxine fails also to abolish the rise of blood pressure produced by sympathin (fig. 4).

The source of sympathin is of importance in these observations after

ergotoxine or yohimbine. When sympathetic nerves inducing predominantly or exclusively excitatory effects are stimulated, such as the cardiac or the hepatic nerves, the rises described above ensue. Stimulation of mixed sympathetic nerves, such as the superior mesenteric or the hypogastrics, which induce both excitatory (vasoconstriction) and inhibitory (relaxation of the g.i. tract, the bladder and the non-pregnant uterus) responses, may produce a fall of blood pressure, as does adrenine (cf. Rosenblueth and Cannon, 1935). These falls are more readily obtained after large doses of ergotoxine or in unanesthetized spinal animals, in which the constrictor influence of adrenine is reversed by smaller doses of ergotoxine, than in animals under dial anesthesia (cf. Cannon and Rosenblueth, 1933).

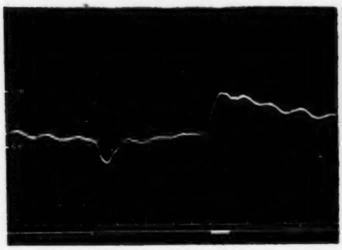


Fig. 3

Fig. 4

Fig. 3. Dial, cocaine (8 mgm. per kgm.) and yohimbine (3 mgm. per kgm.). Adrenals tied off. Hepatic nerves cut. Effects of injecting 0.1 cc. adrenine (1:100,000) and of stimulating the right splanchnic (coil distance, 6 cm.).

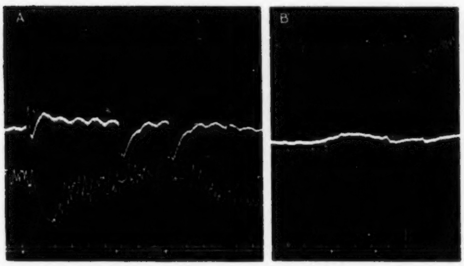
Fig. 4. Dial, cocaine (8 mgm. per kgm.), yohimbine (3 mgm. per kgm.) and ergotoxine (2 mgm. per kgm.). Adrenals tied off. Effects of stimulating the hepatic nerves (coil distance, 6 cm.) and of injecting 0.3 cc. adrenine (1:100,000).

The effects of sympathin on the pregnant uterus. Whether sympathin is derived from cardiac, hepatic or gastro-intestinal sources it induces a contraction of the cat's pregnant uterus, as does adrenine. Figure 5 illustrates these responses.

The influence of acetylcholine and atropine on the non-pregnant uterus. Adrenine is known to relax the cat's non-pregnant uterus. Sympathin from certain sources (gastro-intestinal, cardio-pulmonary) has been shown to induce a similar relaxation, while that of hepatic origin fails to evoke this relaxation (Cannon and Rosenblueth, 1933). Acetylcholine in doses sufficient to elicit marked blood-pressure falls is without influence on the non-pregnant uterus (fig. 6A), even after eserine. Large doses after atropine are likewise ineffective (fig. 6B). Adrenine, on the other hand, is still active after atropine (fig. 6B), and so is sympathin from a distant source or that produced locally on stimulation of the hypogastric nerves (fig. 7).

Summary of the effects of sympathins from different sources and of adrenine on various indicators. We have now at hand data concerning the responses of a considerable variety of organs to sympathin and adrenine, that reveal

Fig. 5. Spinal. Curare. Cocaine. Record of the pregnant uterus. Effects of injecting 0.2 cc. adrenine (1:200,000) and of stimulating the hepatic nerves (coil distance, 6 cm.).



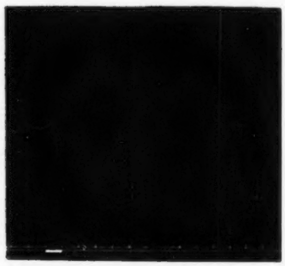


Fig. 6 Fig. 7

Fig. 6A. Spinal. Curare. Cocaine. Upper record, blood pressure. Lower record, non-pregnant uterus. Effects of injecting 0.1 cc. adrenine (1:100,000), 0.05 and 0.1 cc. acetylcholine (1:2,000,000).

B. Spinal. Curare. Atropine (1 mgm. per kgm.). Upper record, non-pregnant uterus. Lower record, blood pressure. Effects of injecting 0.1 cc. acetylcholine (1:100,000) and 0.1 cc. adrenine (1:100,000).

Fig. 7. Spinal. Curare. Atropine (1 mgm. per kgm.). Response of the non-pregnant uterus to stimulation of the hypogastric nerves (coil distance, 7 cm.).

significant relations. These data are presented in tables 1 and 2. Other sources of sympathin than those listed have been studied (e.g., the hypogastric nerves, the superior cervical sympathetic); and other indicators have been observed (e.g., the retractor penis, the submaxillary gland).

The data are, however, not sufficiently extensive to be included in these tables. It is of interest to note that cocaine increases *all* the effects mentioned in the tables.

In the tables are represented sources of sympathin E and I—the gastro-intestinal tract and the regions innervated by the lower abdominal sympathetic chains and the cardio-pulmonary nerves; and a source of sympathin E alone—the area of distribution of the hepatic nerves (cf. Cannon and

TABLE 1

Comparison of the effects of sympathin and adrenine on various indicators (in the cat), and under various experimental conditions

Double signs (++ or −−) signify greater effects than single signs. G.I. indicates the gastro-intestinal tract; L.a.s., the lower abdominal sympathetic distribution; C.a., the cardio-accelerator distribution; and H.ns., the hepatic nerves.

INDICATORS	ADRENINE	SYMPATHIN			
		G.I.	L.a.s.	C.a.	H.ns.
Heart rate	+	+	+		+
Blood pressure	Small doses − Large doses +	+	+	++	++
Leg vessels	Small doses − Large doses +	+			++
Nictitating membrane	+	+	+	+	+
Pregnant uterus	+			+	+
Non-pregnant uterus	−, or − → +	−, or − → +		−, or 0	0
Pupil	++			±	±

TABLE 2

After ergotoxine or yohimbine (same abbreviations as in table 1)

INDICATORS	ADRENINE	SYMPATHIN			
		G.I.	L.a.s.	C.a.	H.ns.
Heart rate	+	+	+	+	+
Blood pressure	−	− or +	− or +	+	++

Rosenblueth, 1933). Also are represented indicators having both a contractile and an inhibitory sympathetic supply (e.g., leg vessels) or only a single supply, contractile (e.g., nictitating membrane) or inhibitory (e.g., non-pregnant uterus). Examination shows that strict concordance between the actions of sympathin and adrenine obtains in the denervated heart, nictitating membrane and pregnant uterus—structures which have single sympathetic (excitatory) innervation. Where there is a dual sympathetic supply—the general arterial system, represented by "blood

pressure" and "leg vessels"—small and large doses of adrenine have different effects, which theoretically might be duplicated if we could evoke sympathin I separate from sympathin E. Since that has not yet been achieved, however, there is here some discrepancy between adrenine and sympathin. The most marked discrepancy between them is found in their effects on the non-pregnant uterus, which has a single sympathetic (inhibitory) supply. If sympathin I should reach this uterus it would have the relaxing influence of adrenine itself; but sympathin E would have no effect. Sympathin from a dual source, therefore, acts like adrenine, while sympathin from a single (excitatory) source is inactive. It is noteworthy that in comparison with all other indicators the iris, with its antagonistic muscles, stands quite apart (see Cannon and Rosenblueth, 1935).

DISCUSSION. *Important differences between sympathin and adrenine.* In recent literature on the mediation of sympathetic nerve impulses the effects similar to those produced by adrenine have been designated "adrenergic" (Dale, 1934). In suggesting this term Dale was careful to disclaim any intention to prejudge the nature of the chemical mediator. Because the word *adrenergic* is likely to suggest, however, that sympathin and adrenine are the same, and because the view has been advanced that they are nearly if not quite identical (cf. Bacq, 1933), we wish to summarize briefly here the evidence that they are different. 1. Sympathin E causes a rise of blood pressure after ergotoxine or yohimbine, adrenine causes a fall (figs. 1, 2, 3 and 4; table 2). 2. Sympathin E is ineffective on the non-pregnant cat uterus, adrenine causes relaxation (Cannon and Rosenblueth, 1933). 3. Sympathin E stimulates the nictitating membrane, but does not cause dilatation of the pupil; adrenine induces both changes (Cannon and Rosenblueth, 1935). 4. The summed influence of sympathin E from two sources is greater than the summed influence of two equivalent doses of adrenine (Rosenblueth and Morison, 1934). Until these observed facts are set aside, or proved of no significance, sympathin E should not be confused with adrenine.

Sympathin I is not acetylcholine. The existence of sympathetic cholinergic fibers (Dale and Feldberg, 1934; Bülbring and Burn, 1934; Rosenblueth and Cannon, 1935) might suggest that sympathin I could be acetylcholine of sympathetic origin while sympathin E could be an adrenine-like substance with only excitatory action. In the paper mentioned we have given reasons for regarding the fall of blood pressure which may occur on stimulation of sympathetic nerves after ergotoxine and atropine as due to a non-cholinergic sympathin I. The data on the non-pregnant uterus are furthermore conclusive in this respect; acetylcholine does not induce relaxation, either before or after atropine (fig. 6), while adrenine does induce it (fig. 6), as does stimulation of the hypogastrics, whether atropine be present or not (fig. 7). We therefore conclude that there is a sympathin

I—i.e., an "adrenergic" product of sympathetic nerve stimulation—which differs from acetylcholine and does not act by liberating acetylcholine, but possesses relaxing properties of its own.

Defense of the concept of sympathins E and I. Three more or less different substitutes for this concept have been put forward by Bacq (1934).

1. The first alternative (p. 480) is that the sympathetic chemical mediator, M or sympathin, is equivalent to adrenine, that it oxidizes rapidly (presumably on its way from source to indicator, for Bacq stresses the long interval—2 minutes—between the start of stimulation and the start of response), and that by partial oxidation it might lose its inhibitory power. It would then become, in our view, equivalent to sympathin E, excitatory only. This hypothesis does not accord with the fact that sympathin E is obtained from sources (liver, heart) which yield it in large amounts—which would probably be more slowly oxidized than small amounts—and so promptly that the positive effects are produced, not in minutes, but in a few seconds. Thus far sympathin I has been demonstrated most strikingly when derived from the alimentary canal, a source which apparently yields small amounts and from which it passes slowly to the test object—just the condition which, according to Bacq, would be most unfavorable for its appearance. Furthermore, if in partial oxidation adrenine loses its inhibitory action, one would expect that small doses, quickly oxidized, would be purely excitatory; in fact, on blood pressure they have a depressor effect. And finally, if sympathin is the same as adrenine it should act like adrenine after ergotoxine on arterial pressure (cf. Cannon and Rosenblueth, 1933), and on the iris (Cannon and Rosenblueth, 1935), but it does not.

2. The second alternative offered by Bacq (p. 480) involves the postulation of two mediating substances: adrenine where the sympathetic has inhibitory effects, and noradrenine (i.e., non-methylated adrenine) where excitatory. Note that demethylation would be necessary before injected adrenine could exert its excitatory action. And if that process should occur routinely, it would probably occur more rapidly with small than with large amounts. The depressor action of small doses, therefore, would not harmonize with this second suggestion. Also it fails when adrenine and sympathin are compared in their effects on the iris.

3. The third possibility considered by Bacq (p. 481) is that potassium ions liberated when the sympathetic is stimulated would accentuate the excitatory and lessen the inhibitory influence of sympathin. Bacq and Rosenblueth (1934) have shown, however, that when potassium ions are injected they induce contraction of the non-pregnant cat uterus if the adrenals have previously been excluded, but relaxation if not excluded. The relaxation, therefore, is due to medulliadrenal secretion (evoked by the potassium) which may be regarded as overwhelming the contractile action of potassium. If sympathin E were adrenine *plus* potassium ions

it might be expected to make the uterus relax to a marked degree, due not only to the adrenine present in it, but also to the adrenine which the potassium ions evoke from the adrenals. But we know that when either the hepatic nerves or cardio-accelerators are stimulated, in such manner as to produce sympathin E, the nictitating membrane is made to contract and the cat's non-pregnant uterus is not made to relax (Cannon and Rosenblueth, 1933). Bacq's third suggestion, therefore, appears, like the others, to meet insurmountable obstacles.

Another consideration of some importance is that Bacq's three suggestions fail to explain simply and reasonably the *change* in the responses of the cat uterus to adrenine and sympathin, from relaxation when non-pregnant to contraction during pregnancy. The view which we are defending would explain the change as a conversion of the receptor I into E.

Admittedly the existence of sympathin I, apart from sympathin E, has not been demonstrated—a fact explicable by failure to isolate in the organism a purely inhibitory sympathetic nerve supply because vasoconstrictor (excitatory) fibers seem to be everywhere present. Even though sympathin I, different from adrenine, has been postulated on only indirect evidence, however, it offers the sole hypothesis which accounts for inhibition by sympathetic impulses and adrenine. And furthermore, if the existence of sympathin E is granted—for which there is direct evidence—the inhibitory agent, sympathin I, cannot, we are convinced, be pure adrenine. In that case stimulation of a mixed source (e.g., splanchnic stimulation of the gastro-intestinal tract) would yield sympathin E from the contracted and adrenine from the inhibited smooth muscle. But adrenine is both excitatory and inhibitory. In consequence, the mixture would always have an excess of excitatory effect if its inhibitory effect should be matched with adrenine—i.e., if the relaxation of the non-pregnant cat uterus from splanchnic stimulation were equaled by a certain amount of adrenine, that amount could not produce the corresponding contraction of the nictitating membrane, for that would have resulted from sympathin E *in addition to the adrenine.* But it is quite possible to reproduce quantitatively the effects of sympathin, + and −, by the injection of adrenine (see Cannon and Rosenblueth, 1933; also unpublished observations by Bacq, Cannon and Rosenblueth). We therefore conclude that if the existence of the excitatory sympathin E is admitted, the inhibitory chemical mediator is not adrenine, and must be a humoral agent arising from sympathetic stimulation and having special inhibitory power. That would be a good definition of sympathin I.

SUMMARY

The effects of sympathin from various sources—gastro-intestinal tract, hind limbs and tail, heart and lungs, and liver (in cats)—are compared with

the effects of adrenine on the following indicators: heart rate, blood pressure, leg vessels, nictitating membrane, pregnant and non-pregnant uterus, and pupil (table 1). A similar comparison is made of the effects of sympathin and adrenine on the heart rate and blood pressure after yohimbine or ergotoxine (table 2). The data contained in tables 1 and 2 are partly drawn from previous publications and have been completed by the results reported in the present study (figs. 1 to 7). Discussion of these data leads to the following conclusions: a, sympathin differs from adrenine (p. 273); b, there are two sympathins, one excitatory (E), the other inhibitory (I) (p. 274); and c, sympathin I is not acetylcholine (p. 273).

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THE EQUATION OF THE VOLTAGE-CAPACITY CURVE FOR THE EXCITATION OF THE SCIATIC NERVE OF RANA PIPIENS

H. A. BLAIR

From the Department of Physiology, School of Medicine and Dentistry, The University of Rochester, Rochester, N. Y.

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Considerable evidence has been presented of the validity of a certain description, in the form of equations, of the processes of excitation in response to electrical stimuli of various kinds (1932–35). In no case, however, has a large number of excitation curves obtained with one type of stimulus been considered in order to demonstrate the presence or absence of systematic divergences between the data and the equation devised to represent them. It is the present purpose to consider a group of voltage-capacity curves from this point of view.

There is sufficient evidence that any divergences which may exist with the muscles and nerves of the frog, at least, are small, so that as regards the use of the scheme developed as an aid in planning and correlating experiments, the question of whether or not they are systematic is of relatively little importance. It is of importance, however, to consider any systematic divergences when the problem arises of interpreting in physico-chemical terms the equations of the phenomenological scheme, particularly when it is necessary to choose between two or more modes of representation which appear about equally probable and relate the data about equally well.

Another purpose served by determining the equation of any type of stimulus accurately is the establishment of a criterion whereby any hypothesis concerning the excitatory process may be tested easily. For if an equation exists, which has been shown to represent a certain body of data with a certain degree of accuracy, then any hypothesis of probable validity must be representable by an equation which reduces to the first with at least equal accuracy; and in general it is much easier to compare two equations than it is to test one by applying it to a large body of data.

The present purpose is to show the accuracy of representation of a large number of voltage-capacity data from the stimulation of the sciatic nerve of *Rana pipiens* by the equation,

$$\frac{V}{R} = (crk)^{\frac{1}{crk-1}} \tag{1}$$

where V is the peak voltage of the stimulus, R is the rheobase, c and r are the capacity and resistance, respectively, of the circuit, and k is a constant.

This equation is the solution (1932c) of the differential equation,

$$\frac{dp}{dt} = KV - kp \tag{2}$$

where p is the excitatory process and K is a constant, using the conditions that p is initially zero and is adequate at its maximum when its maximum value is equal to $h \pm \alpha V'$, h and α being constants, and V' being the voltage at the time p is a maximum. Appropriate solutions of this equation with the same boundary conditions have been shown to be consistent for direct currents, linearly rising currents and condenser discharges as stimuli (1935a, b). With condenser stimuli the solution of equation 2 is the same, i.e., equation 1 is the same, whether the threshold is $h \pm \alpha V'$, or simply h. Consequently k is the only parameter whose value is obtainable by means of equation 1.

Experimental method. All the data were obtained from sciatic-gastrocnemius preparations of *Rana pipiens* which had been kept in cold Ringer's solution from one to several days after dissection. The nerve was suspended in air on the electrodes, which were silver wires 0.5 mm. in diameter lightly covered with the chloride. The electrode separation was from 1 to 2 cm. The stimulating circuit consisted of a battery of 135 volts in series with the condenser and a non-inductive resistance of 50,000 ohms. Part of this resistance was used as a potentiometer from which the stimuli were derived. The resistance of the potentiometer between the leads never exceeded about 1 per cent of the resistance of the tissue. The charging current was used to stimulate and the condenser was discharged between stimuli by short-circuiting. The condensers were of high quality with mica dielectric. The temperatures, which covered nearly the whole physiological range, were maintained constant to about 0.2°C. during the taking of each set of data. The time required to obtain each set averaged about 6 minutes. Each datum is a single determination. The rheobase was measured initially and finally.

Data and method of calculation. In table 1 are given 36 sets of data. These were selected from about 50 by eliminating first those in which the final rheobase differed from the first by more than about 2 per cent. This left 45 sets which were calculated. The 36 most consistent of these were then selected for presentation. Of the remainder, five had rheobases less than 40 units and the consequent inaccuracy of reading made the plotted curves irregular and the evaluation of k difficult. The other four curves had the largest single divergences.

In the table, the first column gives the capacities of the condensers in

TABLE 1

VOLTAGE CAPACITY CURVES AS MEASURED AND CALCULATED ACCORDING TO EQUATION 1

		A			B			C			D			E			F		
	C	V OBS.	V CAL.	V_c/V_o	V OBS.	V CAL.	V_c/V_o	V OBS.	V CAL.	V_c/V_o	V OBS.	V CAL.	V_c/V_o	V OBS.	V CAL.	V_c/V_o	V OBS.	V CAL.	V_c/V_o
	6.0	63	63		42	41		77	77		43	43		37	37		64	64	
	1.0	63	67	1.06	45	43	0.96	79	80		43	45	1.05	38	39	1.03	65	65	
	0.5	68	70	1.03	47	45	0.96	83	90	1.08	43	46	1.07	40	40		68	69	1.02
	0.1	86	87		62	56	0.90	99	100		54	54		48	47	0.98	80	80	
	0.05	104	104		70	67	0.96	117	122	1.04	62	62		55	54	0.98	91	91	
	0.02	145	146		100	93	0.93	157	156		78	80	1.03	72	70	0.97	120	119	
1	0.01	211	217	1.03	130	132	1.02	212	217	1.02	107	105	0.98	95	91	0.96	160	159	
	0.008	237	236		160	150	0.94	240	241		118	122	1.03	104	106	1.02	175	172	0.98
	0.004	372	371		230	234	1.02	361	363		175	177		154	154		260	260	
	0.003	458	458		285	288		442	444		213	212		190	185	0.97	310	314	1.02
	0.002	624	623		392	392		580	592	1.02	283	282		249	246		413	415	
	0.001	1115	1120		636	688	1.08	1032	1029		478	473		428	416	0.97	700	705	
	6.0	63	k = 1390		41	k = 1460		77	k = 1920		43	k = 2400		38	k = 2400		64	k = 2430	
	6.0	47	47		48	47		37	37		41	41		51	50		44	44	
	1.0	47	49	1.04	47	49	1.04	38	38		41	42	1.02	51	51		44	45	1.02
	0.5	49	50	1.02	49	50	1.02	39	39		42	43	1.02	51	53	1.04	45	46	1.02
	0.1	58	58		57	57		47	45	0.96	50	49	0.98	59	60	1.02	51	52	1.02
	0.05	64	66	1.03	65	65		52	50	0.96	57	55	0.96	66	66		58	58	
	0.02	85	85		84	82		67	63	0.94	72	70	0.97	86	83	0.97	72	72	
2	0.01	113	113		107	107		84	82	0.98	92	91		112	106	0.95	92	91	
	0.008	122	124	1.02	119	119		95	87	0.92	101	97	0.98	119	117	0.98	100	100	
	0.004	178	182	1.02	170	172		134	129	0.96	146	143	0.98	165	164		146	140	0.96
	0.003	210	229	1.09	203	206	1.02	157	154		172	170		194	194		164	166	1.02
	0.002	281	288	1.03	260	269	1.03	204	201		222	222		252	253		215	215	
	0.001	482	488		448	452		334	362	1.08	364	401	1.10	412	414		351	352	
	6.0	47	k = 2610		47	k = 2890		37	k = 3140		41	k = 3140		50	k = 3480		45	k = 3660	
	6.0	141	142		39	39		71	70		56	56		40	40		72	71	
	1.0	143	146	1.02	39	40	1.03	71	72		56	57	1.02	41	41		72	73	
	0.5	146	149	1.02	41	41		72	73		57	59	1.03	41	42	1.02	73	74	
	0.1	168	168		46	46		80	82	1.02	65	66	1.02	46	47	1.02	82	83	
	0.05	190	186	0.98	52	51	0.98	93	88	0.95	73	73		52	51	0.98	91	91	
	0.02	237	230	0.97	65	63	0.97	115	112	0.97	92	90	0.98	63	63		110	100	0.91
3	0.01	302	293	0.97	80	80		144	140	0.97	114	113		81	79	0.98	138	140	
	0.008	322	321		89	87	0.98	162	155	0.96	127	124	0.97	89	86	0.97	154	153	
	0.004	460	450	0.98	120	122	1.02	225	217	0.96	177	174	0.98	124	120	0.97	209	212	
	0.003	535	531		140	144	1.03	268	255	0.95	212	203	0.96	145	140	0.97	242	248	1.02
	0.002	695	672	0.97	180	185	1.03	336	328	0.97	263	255	0.97	181	180		307	318	1.04
	0.001	1125	1120		304	303		533	533		428	425		288	290		517	517	
	6.0	143	k = 3720		39	k = 3800		70	k = 3900		58	k = 3930		40	k = 4150		771	k = 4170	
	6.0	62	62		77	77		72	71		67	66	0.98	82	80		63	63	
	1.0	62	63	1.02	77	79	1.02	72	73		65	68	1.05	82	81		63	64	1.02
	0.5	63	65	1.02	79	80		73	74		65	69	1.06	82	84	1.02	65	66	1.02
	0.1	71	72		87	89	1.02	82	82		75	76		94	92	0.98	73	73	
	0.05	78	79		99	98		90	91		79	83	1.05	104	102	0.98	81	79	0.97
	0.02	96	97		120	119		112	109	0.97	104	100	0.96	127	122	0.96	100	96	0.96
4	0.01	123	122		154	149	0.97	138	136		129	125	0.97	155	151	0.97	124	119	0.96
	0.008	133	133		163	163		153	148	0.97	140	136	0.97	167	165		132	130	
	0.004	181	185	1.02	225	222		205	206		187	189		228	228		180	180	
	0.003	215	216		260	262		244	239	0.98	222	218	0.98	261	264		209	208	
	0.002	275	277		329	338	1.03	306	306		272	276		329	335	1.02	257	264	1.03
	0.001	420	446	1.06	514	542	1.05	488	492		433	442	1.02	534	537		416	422	
	6.0	63	k = 4200		77	k = 4360		71	k = 4450		67	k = 4660		80	k = 4660		63	k = 4660	
	6.0	44	44		71	70		69	68		55	54		52	52		56	54	
	1.0	44	45	1.02	71	72		70	70		54	55	1.02	52	53	1.02	55	55	
	0.5	45	46	1.02	71	73	1.03	70	71		55	56	1.02	54	54		55	56	1.02
	0.1	50	51	1.02	79	80		78	78		60	62	1.03	59	59		61	62	1.02
	0.05	56	55	0.98	88	86	0.98	87	82	0.94	66	65	0.98	66	65	0.98	65	67	1.03
	0.02	69	67	0.97	107	105	0.98	105	104		80	81	1.02	80	78	0.98	80	80	
5	0.01	84	83		134	130	0.97	129	126	0.98	100	100		97	95	0.98	98	98	
	0.008	90	90		143	143		144	137	0.95	109	108		107	103	0.96	106	107	
	0.004	122	122		192	188	0.98	193	171	0.89	142	135	0.95	144	141	0.98	140	146	1.04
	0.003	144	144		226	225		223	217	0.97	170	172		172	163	0.95	160	167	1.04
	0.002	181	182		287	286		274	273		213	215		218	205	0.94	210	212	
	0.001	283	290	1.02	455	453		432	435		337	346	1.03	328	326		315	336	1.07
	6.0	44	k = 4730		70	k = 4860		68	k = 4950		54	k = 4970		52	k = 5090		54	k = 5150	
	6.0	61	61		58	57		56	56		87	87		86	86		95	95	
	1.0	61	62	1.02	58	58		56	57	1.02	88	88		87	87		96	96	
	0.5	61	63	1.03	60	59	0.98	57	58	1.02	89	89		87	88		96	97	
	0.1	69	70		65	63	0.97	62	62		95	94		94	93		99	101	1.02
	0.05	75	75		72	68	0.94	69	67	0.97	104	99	0.95	101	97	0.96	104	106	1.02
	0.02	92	90	0.98	84	80	0.95	83	78	0.94	117	112	0.96	113	109	0.96	115	117	1.02
6	0.01	114	109	0.96	100	95	0.95	100	94	0.94	127	128		130	125	0.96	132	133	
	0.008	123	118	0.96	110	105	0.95	107	102	0.95	145	137	0.94	139	132	0.95	138	140	
	0.004	163	160	0.98	142	135	0.95	138	133	0.96	178	171	0.96	166	164		168	172	1.02
	0.003	188	185	0.98	161	156	0.97	159	153	0.96	204	192	0.94	193	184	0.95	187	191	1.02
	0.002	236	234		195	193		194	189	0.97	240	232	0.97	220	219		220	227	1.03
	0.001	365	368		293	297		290	292		355	338	0.95	308	317	1.03	300	321	1.07
	6.0	61	k = 5380		57	k = 6650		56	k = 6650		87	k = 10500		86	k = 11350		95	k = 13200	

C IN MICROFARADS; UNIT V = 27×10^{-4} VOLT; RESISTANCE = 50,000 OHMS.

microfarads. The second gives the measured voltages of the stimuli while the third contains the voltages calculated according to equation 1. In the fourth column are the ratios of the calculated to the measured voltages. The remaining columns in each row, in sets of three, are the same as the second, third and fourth. The numbers 1, 2, etc., and the letters A, B, etc., identify the separate curves. The 6 microfarad reading is taken as the rheobase.

The sets of data are arranged in order of increasing k values, that of the least being 1390 and the greatest 13,200. The temperatures at which the readings were made increased in about the same order, those in row 1 having been from 6 to 11°C., in row 2 from 9 to 16°C., in row 3 from 11 to 19°C., while most of the remainder were from 20 to 28°C. The data are representative, therefore, of the physiological temperature range and as a consequence of the normal range of the values of k, for the most easily stimulated nerve elements of the trunk.

The extent of the curves, i.e., the number of rheobases to which they extend, is variable because no capacity less than 0.001 microfarad was used. Consequently curves of large k are shorter than those with small, so that the last set of data goes to only three rheobases, while the first goes to about seventeen. The intermediate sets extend on the average to about eight rheobases.

The method of calculation has been given before (1932c, 1934). The constant k was determined as usual from the linear relation,

$$cr \log \frac{V}{R} = \frac{1}{k} \left\{ \log \frac{crV}{R} + \log k \right\} \tag{3}$$

which is the same as equation 1. In calculating the quantities $cr \log V/R$ and $\log crV/R$ the smallest measured rheobase was used if the initial and final values were not equal. The line determined by these quantities should, according to equation 3, have a slope of $1/k$ and an intercept on the abscissa, $-\log k$. The mean value of k from these two sources was used to calculate V, the measured values of R and cr being assumed correct. Actually in the method, the quantity k is obtained from the data for the smaller half of the capacities because the line determined using voltages too near the rheobase is too sensitive to small variations.

It will be noted that a systematic divergence is introduced by the method on account of the smallness of the rheobase which is often about 50. This number must always be greater than or equal to the real value which will lie between the measured value and one unit less than the measured value. The measured value of the rheobase, therefore, which has been assumed to be correct actually is always too great by an amount which may be as much as 2 per cent, approximately. On the other hand, the highest voltages are measurable with greater accuracy because they are

in the hundreds. As a consequence of these facts, the quantities V/R of equation 3 will be usually somewhat too small and k, therefore, will be somewhat too large. On account of k, the curvature of the theoretical curve will be somewhat too great although the method makes it match the measured curve at the ends. In all cases, therefore, in which the measured rheobase is too high the calculated voltages will tend to be somewhat too low in the middle of the curve and too high near the rheobase. This divergence should not be great but it will be systematic.

Another feature of the method to be noted also in connection with the rheobase, is that it has been assumed to be correct in finally calculating V. Referring to equation 1, it will be seen that even if k has been correctly determined, and if the right hand of equation 1 has been calculated correctly, an error will occur in V when it is obtained by multiplying the right side of equation 1 by R, if R is too great or too small. The effect of this will be to make all the calculated values of V too great or too small in the same ratio as R is too great or too small. This effect could be adjusted by choosing a theoretical rheobase which gave the best fit, but this was not done in the present case, except that in three instances the mean of the initial and the final rheobases was used instead of the smallest one.

It must be emphasized that these data for the reason given above, and because the temperature was not controlled very closely and because the measurements were not repeated, are probably not as exact as they might be. Any improvement except in regard to temperature would probably require a greater time in taking each curve, but this would not likely be serious as the preparations are very stable. The lack of repetition of measurements, in particular, although saving in time, allows possible occasional large errors in reading the voltage to pass unnoticed, and for this reason it seems certain that data could be obtained whose large errors in measurement were much reduced, and it is altogether likely that the readings near the rheobase could be made somewhat more exact by the use of a finer scale of voltages. The present data have advantages, however, in that they cover a large range of temperatures, are numerous, and are routine measurements not highly selected, so that they will not only test the validity of equation 1 but will indicate also how closely ordinary measurements can be expected to conform to it.

Discussion. It is difficult to form an estimate of the real errors of observation since the tissues are subject to variation. It is indicated by the agreement of the initial and final rheobases, however, that, in the cases given, this variation is usually within the limits ± 2 per cent. The rest of the curve may vary independently of the rheobase, however, particularly as the result of temperature changes, so that the rheobase agreement is not necessarily an adequate index. As regards the apparatus, the series resistance was kept adjusted to 50,000 ohms within ± 0.2 per cent. This

variation and that of the tissue will not be systematic so that in their regard the variations should lie within the range of ± 3 per cent. The shunting of the potentiometer by the tissue may introduce with high voltages a systematic positive error in the readings not exceeding 1 per cent. The condensers were factory standardized to at least ± 2 per cent with the smallest capacities, the remainder being better. Apart then from the systematic instrumental errors which will exist only with the smallest capacities, it may be expected that the bulk of the observations will be correct to ± 3 per cent and the data will be interpreted on this assumption.

It will be seen from table 1 that the measured and calculated voltages are in close agreement. In order to make comparison easier, all the ratios of calculated to measured data which were less than 1.5 per cent greater or less than unity were omitted from the ratio columns. Such divergences may in any case be considered to be within the limit of accuracy of the apparatus and method of calculation. Ratios greater than 1.015 and less than 1.025 are given as 1.02 and so on.

In figure 1 is given a graphical summary of the divergences. The heights of the blocks give the percentage of the observations which fall within different groupings of percentage variation from the theoretical curve. The ranges of the variations, 0, 0–1.5, etc., are given at the tops of the blocks. The positive variations are toward the right, the negative toward the left. It can be verified roughly that 48.4 per cent of all the observations lie within ± 1.5 per cent of the theoretical curve, 69.4 per cent lie within ± 2.5, and 81.8 per cent lie within ± 3.5 per cent. The mean of the last two values, 75, will give, approximately, the percentage of the data which lie within ± 3 per cent of the curve, which has been estimated above to be about the range of experimental error. This could be improved somewhat by adjusting the rheobases in some cases, for there are 7 curves in which all the divergences are positive or zero, and 4 in which they are negative or zero. It is evident that in these cases the divergences could be made smaller by adjusting the theoretical curve so that the errors were both positive and negative.

In table 2 is given a summary of the divergences referred to corresponding parts of the curves on a scale of rheobases. These parts are taken as those from 1–1.5 rheobases, 1.5–3.0 rheobases, etc., as indicated in the last column of the table. The first column gives the number of positive divergences greater than 1.5 per cent in each category and the second their sum. The third and fourth columns give similarly the negative divergences and their sum. The fifth column contains the numbers of zero divergences (less than 1.5 per cent) while the sixth gives the total number of observations. In the second last column is given the average divergence which is obtained by dividing the sums of the divergences by the total number of observations, each zero divergence being given, arbitrarily, the value unity for this purpose.

It will be seen that each category has one-half or more of its observations with zero divergence except that from 1.5–3.0 rheobases. In this case only three-eighths have zero divergence and many more of these are negative than positive, indicating that there is a systematic tendency

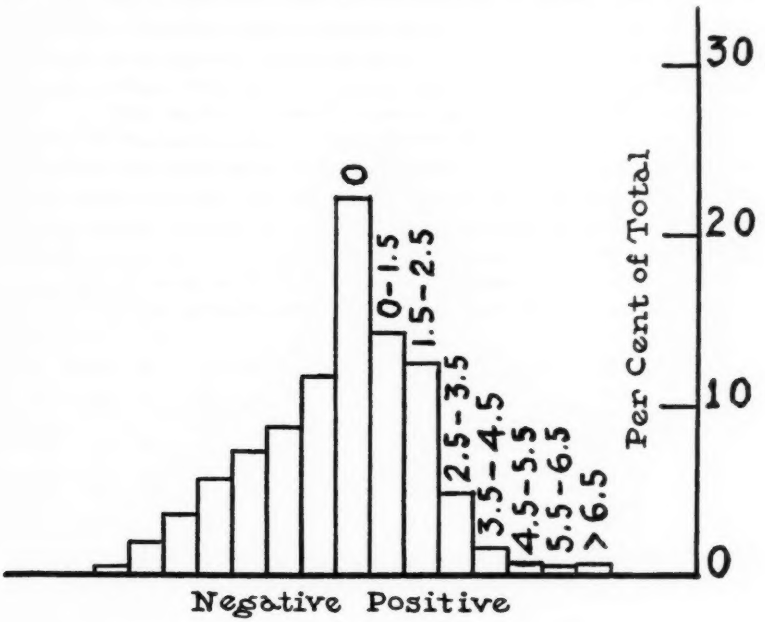

Fig. 1

TABLE 2

The distribution of the divergences related to different parts of the curves on a scale of rheobases

POSITIVE		NEGATIVE		ZERO		TOTAL NUMBER OF OBSERVATIONS	AVERAGE DIVERGENCE	RANGE IN RHEOBASES
Number	Sum	Number	Sum	Number	Sum			
57	155	34	109	97	97	186	1.9	1 -1.5
11	33	66	209	45	45	120	2.3	1.5-3.0
20	62	22	70	42	42	83	2.1	3.0-6.0
10	44	1	3	27	27	38	2.0	>6.0

here for the theoretical curve to fall lower than the observed curve. Since all the curves have been treated in the same way, the average divergence permits an estimation of this discrepancy, because the average divergence in any category indicates approximately the average distance at which

any curve representing the observations could lie from the present curve. Therefore, since the average divergence in each group is about 2, even if the groups on either side of that from 1.5–3.0 rheobases had all their divergences of positive sign while that group had all its divergences of negative sign, the average total systematic divergence in these regions could not differ greatly from the arithmetic sum of the averages, i.e., from 4 per cent. Therefore, even if there is real systematic divergence in the curves, i.e., if this apparent one is not accounted for by the fault, mentioned above, in the method, it cannot greatly exceed the limits ±2 per cent.

The matter may be considered also with regard to the individual curves rather than the group. In table 3 are given the total divergences in each curve. The rows and columns have the same designations as those in table 1. The numbers give the arithmetic sums of the greatest positive and the greatest negative divergences in each curve. It is evident that a fit could be made in each case such that the greatest negative and the greatest positive divergences were equal to each other and therefore to half their

TABLE 3

	A	B	C	D	E	F
1	6a	18	8a	9c	7c	4a
2	9c	4a	16	14b	9c	6b
3	5bc	6a	6	7c	5bc	15c
4	6	8	3a	10	6bc	7a
5	5c	5bc	11b	8c	8	7
6	7c	6	8	6	8c	7

sum as given in the table. It will be seen that there are 27 curves with total divergences of 8 or less, 15 curves with 6 or less, 7 curves with 5 or less, and 3 curves with 4 or less. In each of these groups a fit could be made so that no observation disagreed by more than ±4 or ±3, etc.

Also in table 3, along with the numbers, are the letters *a*, *b* and *c* which mark, respectively, those curves in which at least half the observations in each category of rheobases have zero divergence, in which at least one has zero divergence, and in which at least half diverge by ±3 or less. These cases include, as can be seen from the summary above, 24 curves. There can be but few, if any, of these in which equation 1 does not fit the observations within the estimated experimental error ±3 per cent. Of the remaining curves, all but four have total divergences less than 8.

CONCLUSIONS

It appears possible to conclude that equation 1 containing the single arbitrary constant *k* represents the voltage-capacity curves obtained from the stimulation of the sciatic nerve of *Rana pipiens* within or close to the

limits of the experimental error with the present method. A small systematic divergence is apparent at the part of the curve between 1.5 and 3.0 rheobases, but since a divergence of the same kind is introduced by the method of curve fitting employed, the discrepancy, if real, must be less than that indicated. If at any time a choice is necessary between equation 1 and some other which appears to fit equally well it will be necessary to determine the fit of these equations by more elaborate methods, but this can be done on fewer data taken at the most favorable temperature. Until that time, equation 1 may be used to test any hypothesis concerning the excitatory mechanism, for any such hypothesis, if valid, must, when expressed mathematically, give a form which will reduce to equation 1 to within narrow limits. In addition, the local excitatory process may be studied safely by expressing its variation as a function of experimental conditions in terms of the parameter k. For even if equation 1 is not the true representation of the local excitatory mechanism, the constants of the true representation must be expressible in terms of k and the rheobase alone because these two factors, according to equation 1, enable, within close limits, the reproduction of the voltage-capacity curve. They embody, therefore, all the information obtainable from this curve.

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EFFECT OF A DIET POOR IN SALTS UPON THE GROWTH AND COMPOSITION OF THE INCISORS OF THE RAT[1,2]

MIRIAM F. CLARKE and ARTHUR H. SMITH

From the Department of Physiological Chemistry, Yale University, New Haven

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According to the newer point of view in nutrition, the skeleton is considered as a reservoir of mineral salts, which can be mobilized from this structure with surprising readiness. Inasmuch as the teeth are in many ways similar to bone, there is a question as to whether these organs likewise represent a labile store of mineral salts. It has been demonstrated that the chemical composition of the teeth of rats is relatively constant in the face of deficiencies such as lack of adequate calcium and vitamin D (8, 21, 9, 10, 20). The incisors, which continue to grow both in young and in adult rats, even though the diet may be lacking in tooth-forming material, are thus different from the bones which, although they may continue to grow, may suffer considerable change in chemical composition.

The present study was undertaken to determine to what extent changes occurred in the teeth of rats kept on a ration in which the mineral salts were reduced to a minimum but which was otherwise adequate; and to discover whether the changes, if they occurred, could be reversed by introducing the lacking elements into the diet. Particular attention has been given to the recovery phase in its chemical aspects. The ration used caused a cessation of growth in the experimental animals. Similar dietary restriction has been shown to bring about a distortion of the body weight to body length ratio due to the persistence of growth of the skeleton (22). Accompanying alteration of chemical composition of the bones has also been described (16).

Experimental. Young, rapidly growing male rats from the strain described by Anderson and Smith (2) were used in this study. They were raised on the stock diet (diet 1) until they weighed 100 grams, at which time their average age was 35 days. No rat was used which was older than 40 days at a body weight of 100 grams. Groups of 10 to 20 rats were then caged separately and offered *ad libitum* the low-salt diet (diet 3, table 1).

[1] Some of the data in this paper are taken from a dissertation presented by Miriam F. Clarke in partial fulfillment of the requirements for the degree of Doctor of Philosophy, Yale University, 1933.

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At the termination of a 3, 6 or 12 week period, groups were killed, their upper incisors carefully removed and weighed. Moisture content and ash determinations were made, the latter after desiccation at 90°C. and extraction with alcohol and ether. Two to four pairs of teeth were extracted and ashed together and the average weight and per cent of inorganic residue per pair of incisors were calculated (table 3).

Two types of controls were employed: 1, age controls, littermates of the rats on the low-salt ration, but fed adequate synthetic ration (diet 2, table 1) from the time they weighed 100 grams, for the same periods (3, 6 and 12 weeks); and 2, calorie controls, also littermates, fed a diet containing adequate mineral salts, but restricted in calories (diet 4, table 1). The amount fed to each calorie control rat per day was determined by the amount of diet 3 consumed by the low-salt rats, whose food consumption gradually diminished during the experimental period. In equating the allowance of food energy, due consideration was taken of the non-caloric portion (mineral matter) in diet 4. The growth curves of these calorie controls were midway between those of the age controls and of the low-salt rats; their retardation in growth was due solely to inadequate energy intake, whereas the low-salt rats suffered two deficiencies, namely, energy-producing and inorganic matter.

TABLE 1

Composition of the experimental diets

	DIET 2 "ADEQUATE SYNTHETIC"	DIET 3 "LOW-SALT"	DIET 4 "CALORIE CONTROL"
Washed casein (18)	18	18	16.9
Hydrogenated fat ("Crisco")	27	27	25.4
Corn dextrin	51	55	51.8
Salt mixture (14)	4	0	5.9

N. B. Each rat received daily food accessories as follows: 5 drops cod liver oil, 1 cc. of an 80 per cent alcoholic extract of wheat germ equivalent to 2 grams of wheat germ, 200 mgm. of dried brewer's yeast.

Other groups of rats, after subsisting on the salt-poor ration for the above-mentioned periods, were realimented with an adequate synthetic diet (diet 2) given *ad libitum* for various lengths of time as indicated in table 2. The controls for this part of the experiment consisted of 1, age controls, of the same age as the low-salt rats on the day of sacrifice, fed diet 2 throughout, i.e., for 12, 18 or 24 weeks; and 2, calorie controls, fed diet 4 in restricted amounts for 3, 6 or 12 weeks, then diet 2 *ad libitum* for the same time as were the realimented low-salt rats.

At the termination of the above periods, each rat was killed and the teeth treated as previously described.

The casein used in the diets was repeatedly washed with acidulated water in an attempt to remove the inorganic residue (18). The ash of the low-salt diet, determined by the calcium acetate method (12) was 0.44 per cent. Similar determinations on diets 2 and 4 were 2.78 and 3.59 per cent, respectively. The extremely low concentration of the basic ions constituted the chief deficiency in the low-salt diet. The daily intake of individual ions under similar experimental conditions has been discussed (5, 17).

Results. The incisors of the low-salt rats continued to grow during the period when salts were withheld, but at a subnormal and decreasing rate (table 2). The fresh weight of the teeth of the low-salt rats was 92 per cent that of the age controls at 3 weeks, 84 per cent at 6 weeks and 79 per cent at 12 weeks. The weight of the incisors of the calorie controls, on the other hand, never fell lower than 92 per cent of that of the corresponding age controls during the 12 weeks period. These changes in the weights of the teeth of the experimental animals are not in proportion to the relative loss of body weight of the low-salt and of the calorie control rats. The more pronounced effect on weight of the teeth of the low-salt animals than on their calorie controls must be due to the specific lack of mineral salts in the ration.

After realimentation resumption of growth as indicated by body weight occurred (7). A study of the teeth shows that after the shortest period on inadequate mineral supply (3 weeks) realimentation resulted in such acceleration of growth that after 9 weeks the weight of the incisors equalled that of the age controls; but following the longer depletion periods, the subsequent growth was seriously impaired. The incisors both of the low-salt rats and of the calorie controls of the 3 + 9 weeks group weigh more than do those of their age controls. This acceleration might be expected since the growth of the animal as a whole is increased during refeeding to such an extent that the body weights of the low-salt group and of the calorie control group (restricted diet 3 weeks, realimented 9 weeks) exceed the weight of their age controls. This phenomenon of accelerated growth has been described (13) after growth was suppressed by restricted energy intake; according to these investigators, however, the recovery of body weight after salt deficiency was more likely to be incomplete (15). It will be observed that in the cases where the body weight of the experimental rats exceeds that of their age controls (3 + 9 weeks), the weight of the incisors also exceeds that of the controls; whereas in all other groups both the body weight and the weight of the fresh incisors fall short of the respective values for their age control groups. These slight differences, though consistent, are not statistically significant; only in the 12 + 12 weeks group is the mean incisor weight of the realimented low-salt rats

significantly lower than the mean incisor weight of the age controls (S.R.[3] = 5.4). This correlation between dental development and somatic growth might be expected inasmuch as Addison and Appleton (1) have observed

TABLE 2

Body weight and weight of the incisors

WEEKS ON EXPERIMENT*	NUMBER OF RATS		MEAN BODY WEIGHT	MEAN WEIGHT FRESH UPPER INCISORS
Low-salt experiment				
			gm.	*mgm.*
3 + 0	Low-salt	10	129 ± 8.4†	136 ± 15.7†
	Age control	7	178 ± 11.8	148 ± 6.4
	Cal. control	4	145	140
6 + 0	Low-salt	11	149 ± 12.7	160 ± 12.8
	Age control	7	236 ± 25.7	191 ± 8.8
	Cal. control	4	184	183
12 + 0	Low-salt	19	155 ± 25.8	192 ± 17.7
	Age control	10	326 ± 41.0	242 ± 21.2
	Cal. control	9	183 ± 9.3	222 ± 14.1
Realimentation experiment				
3 + 9	Low-salt	10	313 ± 56.2	237 ± 26.7
	Age control	8	289 ± 44.5	234 ± 18.2
	Cal. control	4	337	268
6 + 6	Low-salt	11	241 ± 36.5	226 ± 20.6
	Age control	8	289 ± 44.5	234 ± 18.2
6 + 12	Low-salt	10	313 ± 49.8	249 ± 16.3
	Age control	7	358 ± 58.8	281 ± 26.9
	Cal. control	4	334	259
12 + 12	Low-salt	19	307 ± 37.3	249 ± 29.1
	Age control	9	411 ± 28.8	298 ± 18.6
	Cal. control	4	364	275

* Number following plus mark indicates weeks of realimentation.
† Standard deviation.

a close relationship between the size of the head and the mandible and the development of the incisors.

[3] S. R. (Significance Ratio) = $\dfrac{M_1 - M_2}{\sqrt{(SE_1)^2 + (SE_2)^2}}$, where *SE* is the Standard Error $\dfrac{S.D.}{\sqrt{N}}$. When SR is greater than 2 the difference is considered significant.

Relatively slight changes in the chemical composition of the incisors occur during the first three weeks on the low-salt diet (table 3). At 6 weeks, however, the moisture content of the low-salt incisors is significantly higher than that of the age controls (S.R. = 5.0), and the ash content is significantly lower (S.R. = 5.6). The differences are much greater at the end of 12 weeks, for the relative and absolute amounts of moisture in the teeth of the low-salt group continue to increase while in both of the control groups there are decreases. The relative amount of ash, on the other hand, decreases while that of the controls is increasing. It should be emphasized, however, that the absolute amount of ash per pair of teeth of low-salt rats increases about 15 per cent (from 66.3 mgm. to 76.1 mgm.)

TABLE 3

Composition of the incisors in the low-salt experiment

WEEKS ON EXPERIMENT	GROUP	WATER CONTENT		ASH CONTENT	
		Weight, mgm.	Per cent*	Weight, mgm.	Per cent†
3 + 0	Low-salt	47	34.8 ± 3.10	66.3	74.6
	Age control	50	33.6 ± 1.12	73.6	75.0
	Cal. control	46	33.1 ± 1.12	70.0	75.2
6 + 0	Low-salt	62	38.7 ± 3.73	71.1	72.5
	Age control	57	30.3 ± 1.32	101.5	76.1
	Cal. control	54	29.7 ± 1.59	97.7	76.3
12 + 0	Low-salt	80	41.7 ± 4.18	76.1	69.2
	Age control	66	26.9 ± 0.79	133.5	77.1
	Cal. control	60	27.0 ± 1.13	123.9	77.0

* Per cent of fresh weight of the incisors.

† Per cent of dry-extracted weight of the incisors.

during the 12 weeks of the salt-poor regime. The corresponding increases in weight of ash in the age controls is 81 per cent; in the calorie controls, 77 per cent. Thus there occurs a deposition of mineral salts at a greatly reduced rate in the growing incisors, despite the fact that the experimental ration is extremely poor in these essentials. The teeth grow at the expense of bone, for in the latter organ demineralization occurs to a considerable extent (6).

The alterations which occurred in chemical composition of the incisors of the low-salt rats were no longer evident after realimentation (table 4). Statistical treatment of the data on percentages of moisture indicates that the values for the realimented low-salt and age-control groups are in no case significantly different. The largest difference, in the 12 + 12 weeks

group, is 2.3 per cent and is within the limits of the experimental error. In every case the ash of the teeth, based on the moisture- and fat-free organs, had returned to values normal for their age. These findings maintain even though the teeth remain smaller in size than those of their age controls (12 + 12 weeks).

TABLE 4

Composition of the incisors in realimentation experiment

WEEKS ON EXPERIMENT	GROUP	WATER CONTENT		ASH CONTENT	
		Weight, mgm.	Per cent*	Weight, mgm.	Per cent†
3 + 9	Low-salt	64	27.0 ± 1.69	130.8	76.0
	Age control	63	26.9 ± 0.69	130.7	76.6
	Cal. control	70	26.6 ± 1.19	150.0	76.2
6 + 6	Low-salt	61	26.8 ± 1.78	124.6	75.6
	Age control	63	26.9 ± 0.69	130.7	76.6
6 + 12	Low-salt	66	26.6 ± 0.86	138.6	76.4
	Age control	70	24.9 ± 2.11	161.5	77.0
	Cal. control	66	25.3 ± 1.04	147.3	76.7
12 + 12	Low-salt	68	27.4 ± 2.01	137.1	76.1
	Age control	75	25.1 ± 1.90	170.9	76.6
	Cal. control	73	26.5 ± 1.10	154.6	77.2

* Per cent of fresh weight of the incisors.

† Per cent of dry-extracted weight of the incisors.

DISCUSSION. The lowered percentages of ash reported here for the incisors of growing rats are of the same order as those reported by Templin and Steenbock (20) for the incisors of adult female rats consuming a ration poor in calcium and vitamin D for about eight months. In their experiments the loss of minerals from the teeth could be prevented by the addition of vitamin D to the diet. Whether the deficient factor be vitamin D or, as in the present experiment, inorganic salts, it appears that the composition of the teeth is much more resistant to change than is that of the bones under similar conditions. Table 5 summarizes the results of five investigators who have analyzed both teeth and bones of rats under a variety of experimental conditions. The incisors cannot be considered a labile source of minerals, particularly when compared to the bones. The present study shows that ash was deposited in the incisors while it was being withdrawn from the bones. Corroborative evidence on this point, supplied by a histological study of the teeth and jaw bone, will appear in a later paper (3).

It has been shown (4, 10, 11) that the pathological condition in the incisal dentin of rats fed rachitogenic diets, can be cured by feeding vitamin D. Similar experiments (23) have shown that vitamin A fed after a

TABLE 5

Extent of change in ash content of incisors and bones of rats, found by various investigators

	ANIMAL USED	REDUCTION IN ASH CONTENT	
		Incisors	Bones
		per cent	*per cent*
Present study	Young ♂ rats	7.9	24.2*
Karshan and Rosebury (11)	Young rats	6.1	22.7
Templin and Steenbock (19, 20)	Adult ♀ rats	8.9	10.0
Gies (8)	Young rats	8.5	20.0
Toverud (21)	Adult ♀ rats	3.5	13.2

* Details to appear in a later paper.

period when the vitamin was withheld, restored the incisors to normal. These studies have indicated that histological changes which occur in the incisors as a result of dietary measures, are slight compared with parallel changes which occur in the bones, and are readily repaired. The chemical analyses reported herewith support this fact.

SUMMARY

Rats 35 days of age (100 gm.) were fed a diet in which the limiting factor was lack of mineral salts. Marked restriction of somatic growth resulted. The fresh weight of the incisors was affected less than one would expect judging from the general effect on body weight. During the twelve weeks on the low-salt regime the moisture increased relatively and absolutely and the ash decreased relatively in the incisors. The changes in composition reported are completely compensated by refeeding the same diet in which 4 per cent adequate mineral salt mixture has been incorporated, although with the longest salt deficiency period (12 weeks) the fresh weight of the incisors never returned to control values. The ash of the incisors is relatively stable compared to that of the bones: ash is deposited in the growing incisors while it is withdrawn from the bones, under the adverse dietary conditions used in these experiments.

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THE UTILIZATION OF CARBOHYDRATE DURING AEROBIC ACTIVITY IN ISOLATED FROGS' MUSCLE

C. L. GEMMILL

From the Department of Physiology, Johns Hopkins University, School of Medicine, Baltimore, Md.

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The carbohydrate changes in isolated muscle have been studied extensively under anerobic conditions. Meyerhof (1920) found that the glycogen content of ten grams of muscle decreased when the muscle was stimulated sixty times per minute for one-half hour. Groups of muscles stimulated similarly and then allowed to remain in air for twenty-three hours at 14° showed a reformation of carbohydrate and an increased oxygen consumption for that period of time. Many other experiments have been made correlating the lactic acid formation with muscular contraction under anaerobic conditions. From experiments of this type, conclusions have been drawn that the combustion of carbohydrate gave the energy for muscular contraction and that the same cycle of events took place in aerobic as well as anaerobic conditions. For example, Hill and Kupalov (1929) assumed that the combustion of glycogen was the sole source of energy for the sartorius muscle contracting slowly in oxygenated Ringer's solution. From the tension developed, they calculated that this preparation might oxidize as much as one per cent glycogen.

In contrast to the many experiments made on muscles working under anaerobic conditions or stimulated at such a rate that lactic acid is formed even in an atmosphere of oxygen, few attempts have been made to correlate the disappearance of carbohydrate with muscular activity under strictly aerobic conditions. Hill (1928, 1929) has supplied the theoretical and practical data for such conditions. Ochoa (1930), in Meyerhof's laboratory, reported a series of experiments on the oxygen consumption and the carbohydrate content of muscles stimulated at the rate of one stimulus per minute for eighteen to thirty hours. He found that there was sufficient carbohydrate in the normal muscles to account for the calculated disappearance of carbohydrate. However, if the sugar content was reduced by insulin convulsions, the total energy developed was greater than could have been supplied by the complete oxidation of the available carbohydrate in the muscle. The final values for the carbohydrate content of the muscles were not determined but were calculated from the

$Km_{(O_2)}$ ratio obtained in a different series of experiments. Meyerhof (1931) reported similar experiments in which the semi-membranosis was stimulated for nineteen to forty-six hours in oxygenated Ringer's solution. He determined the initial and the final values of the carbohydrate content of these muscles and found that the normal muscles obtained their energy from the oxidation of sugar. However, if muscles had a low sugar content, they might burn other materials for the energy of muscular contraction. In this work, Meyerhof did not report any experiments in which a pair of resting muscles were kept for forty-six hours to see if they had the same content of carbohydrate at the end of that long period of time. Unless that can be proven, it is impossible to take one muscle for the base line and to conclude anything from the variation in content of carbohydrate in the stimulated muscle. Also, Meyerhof did not determine the oxygen consumption of this preparation in order to obtain the total energy exchange.

In view of the lack of data on the total energy used and the carbohydrate disappearance in isolated muscles during aerobic activity, experiments were designed to measure and to compare these two variables in a resting and a stimulated sartorius muscle from the same frog. These experiments would offer proof as to whether the oxidation of carbohydrate was the sole source of energy for muscular contraction in the isolated muscle under aerobic conditions.

Methods. The method for determining the oxygen consumption was the same as that used by Gemmill (1934) in his study of the respiratory quotient of the frog's sartorius muscle during activity. The total fermentable carbohydrate was obtained by the method described by Cori and Cori (1933).

The first series of experiments was designed to see whether the content of carbohydrate and the utilization of oxygen were the same in two resting sartorii. Three experiments were made in which the sartorii were removed and immediately placed in ice cold sulfuric acid. Another series of determinations was carried out in which the muscles were shaken with the vessels in the water bath for three to six hours before analysis. Following these two series, another group of determinations was made in which one muscle was stimulated with maximal make shocks at the rate of approximately nine per minute for periods of 3.00 to 6.35 hours. The tension was recorded by observing the deflection of a beam of light on a ground glass scale.

Results. The results on the resting muscles are given in table 1. In the three experiments in which the muscles were taken directly for analysis, close agreement was obtained for the fermentable carbohydrate of the corresponding muscles. Greater deviations were observed in the content of carbohydrate in two muscles shaken for periods of three to six hours with the vessels in the water bath, the greatest variation being 0.8 mgm.

per gram of muscle. Therefore deviations of less than this amount would not be significant in determinations of the carbohydrate utilization during stimulation. The average resting oxygen consumption of the muscle attached to the frame was slightly higher than the oxygen consumption of the muscle lying free in the vessel. This was due to the fact that the muscle attached to the frame was under an initial tension which increased the basal oxygen consumption (Feng, 1932). In some of these experiments on resting muscles, two were placed in each vessel since they were very

TABLE 1

Experiments on resting muscles

DATE	NUMBER OF MUSCLES IN EACH VESSEL	DURATION OF EXPERIMENT	OXYGEN CONSUMPTION		TOTAL FERMENTABLE CARBOHYDRATE		CARBOHYDRATE DIFFERENCE	WEIGHT OF MUSCLES	
			I	II	I	II		I	II
1935		*hours*	*mm.³ per gram/hour*	*mm.³ per gram/hour*	*per cent*	*per cent*	*mgm. per gram*	*grams*	*grams*
Jan.									
10	2				0.42	0.46	0.4	0.102	0.096
11	1				2.86	2.90	0.4	0.139	0.134
12	2				2.16	2.12	0.4	0.176	0.166
15	2	5.0	27.0	29.9	2.58	2.58	0.0	0.147	0.154
16	2	5.66			2.72	2.68	0.4	0.140	0.140
18	1	3.0	39.2	39.4	1.48	1.40	0.8	0.179	0.184
22	1	4.0			1.62	1.67	0.5	0.111	0.104
25	2	3.0	23.0	46.2	2.09	2.16	0.7	0.188	0.168
28	2	5.5	31.3	34.2	1.57	1.60	0.3	0.142	0.130
30	2	6.0	20.2	26.4	1.55	1.63	0.8	0.174	0.157
31	1	6.0	22.6	26.5	2.80	2.81	0.1	0.187	0.168
Averages..................			27.2	33.8					

small. When this was done one muscle from each frog was used in a vessel.

The results obtained on stimulating the muscles are given in table 2. The average oxygen consumption rose from a resting value of 25.5 mm.³ per gram per hour to 606 mm.³ in the stimulated muscle. In a former series, Gemmill (1934) obtained an average value of 275 mm.³ per gram per hour for muscles stimulated seven times a minute. The muscles in the present series contracted at a faster rate; they consumed more oxygen and developed more tension per gram of muscle per hour. However, the ratio between tension developed and oxygen consumed, $Km_{(O_2)}$, was of the same

order of magnitude in the two series. The present determinations gave an average value for the $Km_{(O_2)}$ of 1300 while the former series averaged 1183.

The carbohydrate content of the stimulated muscle decreased in each experiment. Since the amount of carbohydrate in the stimulated muscle was compared with that in a resting muscle which had been shaken for a similar period of time, it was not necessary to subtract any calculated

TABLE 2

Experiments on stimulated muscle

DATE	DURATION OF EXPERIMENT	OXYGEN CONSUMPTION		TOTAL FERMENTABLE CARBOHYDRATE		CARBOHYDRATE DIFFERENCE			TENSION	$Km_{(O_2)}$	WEIGHT OF MUSCLES		LENGTH
		R	W	R	W	Actual	Calculated from O_2	Per cent			R	W	
1935	*hours*	*mm.³ per gram/hour*	*mm.³ per gram/hour*	*per cent*	*per cent*	*mgm. per gram*	*mgm. per gram*		*kgm. per gram/hour*		*grams*	*grams*	*cm.*
Jan.													
23	3.17	41.8	668	0.87	0.75	1.2	2.83	42	279	1200	0.129	0.095	4.1
26	6.35	27.8	657	2.12	1.80	3.2	5.67	56	259	1240	0.116	0.110	4.5
Feb.													
1	6.25	24.1	566	1.94	1.75	1.9	4.74	40	276	1463	0.113	0.101	4.3
2	6.00	28.8	(238)*	2.19	2.15	0.4	1.92	21			0.140	0.138	
3	6.0	16.9	578	1.35	1.30	0.5	4.64	11	199	1080	0.112	0.108	4.3
4	6.0	27.2	628	2.40	1.96	4.4	5.04	87	252	1235	0.138	0.112	4.4
6	5.0	14.5	652	2.20	1.91	2.9	4.36	66	299	1355	0.104	0.096	4.2
8	6.0	12.8	588	2.57	2.51	0.6	4.73	13	281	1500	0.131	0.121	4.5
Jan.													
24	3.0	33.6	(391)*	3.16	2.73	4.3	1.57				0.172	0.180	
29	6.33	27.4	507	1.20	0.67	5.3	4.30		224	1270	0.089	0.079	4.1
Averages...		25.5	606					42	266	1300			

* Not included in general average because tendons broke.

value for the carbohydrate used under basal conditions. Also, if there were any gluconeogenesis, the amount of carbohydrate formed would probably be the same in each muscle. Therefore, changes in carbohydrate content may be attributed to the stimulation. When the actual decrease was compared to the theoretical decrease of carbohydrate as obtained from the oxygen consumption, in only two of the ten experiments was the breakdown of carbohydrate sufficient to account for the total energy exchange. However, these two experiments were not trustworthy. In

the experiment of January 24, the tendon broke at the pelvic end of the muscle, causing damage to the muscle. During the experiment of January 29, the belt slipped off the motor and the apparatus was not shaken for an unknown period of time. The tissue was stimulated during this period and may not have received sufficient oxygen. In the experiment of February 2 the tendon at the tibial attachment broke so the total tension was not recorded in this experiment. Breakage at this end does not damage the muscle as does pulling out of the tendon at the pelvic end. In all of the experiments, except those of January 24 and 29, the actual breakdown of carbohydrate cannot account for the total energy exchange as calculated from the oxygen consumption. The average of the ratio between these two variables was only forty-two per cent. The unfermentable part of the total carbohydrate was small. The average for the resting muscles was 0.18 mgm. per 100 grams of muscle while for the stimulated muscles it averaged 0.27 mgm. This change was so small that it would not affect the general conclusions if it were neglected.

Other factors may account for part of the difference between the actual and the theoretical breakdown of carbohydrate and also for the deviations of this ratio between experiments. Some of these variations were due to experimental error. In the resting series there were deviations of carbohydrate contents between two muscles from 0.0 to 0.8 mgm. per gram of muscle. If the same deviations occurred in the experiments on stimulated muscles they might account for 16 per cent of the variations between experiments.

Another part of these differences might be due to a change in the water content of the stimulated muscles. The wet and dry weights of resting and stimulated sartorii were determined. The water content of thirteen resting muscles shaken in Ringer's solution for seven to nine hours varied from 83.0 to 87.4 per cent. A series of nine experiments in which the muscles were stimulated from 2.7 to 5.5 hours with seven stimuli per minute gave results varying from 83.3 to 86.6 per cent. Since the two series deviated within the same limits, it would be impossible to apply an absolute correction to the results obtained in the present work. These variations, however, would account for some of the differences between experiments.

It has been suggested that the carbohydrate molecule might be partially oxidized without varying its total reducing or fermenting values. Also, carbohydrate might undergo a change in its reducing properties without oxidation. These changes might be intensified by stimulation and produce some of the variations noted in and between experiments. The application of these remote possibilities to the present problem must await the isolation and identification of all the sugars present in resting and working muscles.

The remainder of the deviations between experiments are unaccounted for at the present time. Muscles may oxidize mixed metabolites with variable ratios at different periods during stimulation.

DISCUSSION. Lundsgaard (1930) stimulated muscles poisoned with monoiodoacetic acid in nitrogen and oxygen. He found that more tension was developed and that there was less breakdown of phosphogen when the muscles were contracting in oxygen. Hill and his associates (1931) have reported functional recovery for poisoned muscles in oxygen. This fact suggested that other materials than carbohydrate might supply the energy for aerobic contraction in isolated muscles. The results obtained by Ochoa (1930) and Meyerhof (1931) quoted above indicated also that muscles with low carbohydrate content may derive part of their energy for contraction from non-carbohydrate sources.

Gemmill (1934) reported that the respiratory quotient of an isolated muscle rose on stimulation from a resting value of 0.80 to 0.90. He concluded that this rise indicated that the energy for contraction in a "steady state" was not obtained exclusively from the oxidation of carbohydrate. The direct chemical comparison of the total energy expended for contraction and the utilization of carbohydrate in muscles contracting under aerobic conditions has shown this to be the case. The energy for contraction in an isolated muscle comes only in part from the oxidation of carbohydrate.

In Meyerhof's laboratory, Gemmill (1932) showed a direct proportionality between the lactic acid produced and the tension developed in a sartorius muscle stimulated slowly in oxygen-free Ringer's solution. This proportionality was found to hold for values of lactic acid as high as 1.2 per cent. In the present series, no relationship was found between the aerobic breakdown of carbohydrate and tension developed by the muscle. Therefore, it is very probable that chemical events occurring in aerobic contractions may be entirely different from those taking place under anaerobic conditions. It could be assumed, however, that a mixed metabolism was used to reconvert lactic acid into glycogen. Experiments of the present type, however, tell nothing concerning the chain of chemical processes taking place before the final oxidations. Much more work will be needed before the chemical changes occurring during aerobic and anaerobic activity can be coupled into a satisfactory explanation of muscular contraction.

SUMMARY

A comparison was made of the oxygen consumption and the utilization of total fermentable carbohydrate during activity of frogs' isolated sartorii. The muscles were stimulated nine times a minute for periods of three to six hours in oxygenated Ringer's solution. The average utilization of carbohydrate accounted for only 42 per cent of the total energy

exchange as obtained from the oxygen consumption. Therefore other material than carbohydrate is oxidized to supply the energy for contraction of isolated frogs' muscle under aerobic conditions.

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EVIDENCES OF AN ALTERED TISSUE STATE IN VENTRICULAR FIBRILLATION

N. D. KEHAR AND D. R. HOOKER

From the Department of Physiological Hygiene of the Johns Hopkins University

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The present paper makes three specific contributions pointing to an altered tissue state of physico-chemical nature in ventricular fibrillation; *a*, lowering of the freezing point; *b*, liberation of potassium, and *c*, modification of microscopic structure. All the results reported are based upon observations of the isolated dog heart perfused with Locke solution. Fibrillation was induced by tetanic stimulation of the ventricles and recovery from fibrillation with return of a normal sequential rhythm was obtained by the injection of about 2 cc. of 3 per cent KCl into the perfusion cannula.

Freezing point. Pieces of ventricular tissue of approximately equal size, ± 10 grams in weight, were cut from the same heart, 1, after perfusion for 15 minutes; 2, after 5 minutes of fibrillation during which perfusion was continued, and 3, after a second period of perfusion for 15 minutes subsequent to a recovered normal beat obtained by the use of strong KCl. The pieces were blotted dry and placed in small vials with rubber caps which were submerged in an ice-salt mixture.

The freezing point was determined by the thermocouple method devised by Karrer and Estabrook (1) to whom we are indebted for assistance in its application. The thermo-junction of iron and constantan in a no. 24 hypodermic needle was thrust through the rubber cap and buried near the center of the tissue mass. The other similar junction of heavier wire was kept at 0°C. in a Dewar flask, the two junctions being connected through a galvanometer which registered a deflection of 6.5 cm. for each 1°C. This method has particular merit because slight injury is done to the structure of the tissue. In this manner cooling curves were obtained for each piece of tissue as shown in figure 1. Six such experiments were performed, all in substantial agreement.

The curves show common characteristics, a brief super-cooling which could usually be stopped by slight jarring of the vial, a rise to a plateau and a subsequent fall. When the super-cooling is disturbed solidification of the tissue begins and the heat given off thereby stabilizes the temperature and produces the plateau which is taken as the freezing point. As

the curves shown in figure 1 indicate the freezing point of the fibrillated tissue is appreciably below that of the control and recovered specimens, the latter two tending to be of the same value. This result means that in the state of fibrillation the inorganic constituents of the tissue are in a relatively disaggregated condition.

Liberation of potassium. In a search for correlating evidence to accord with the fore-going observations analyses were made of the out-flowing perfusate before and after the establishment of ventricular fibrillation. These analyses concerned two groups of inorganic substances. First those known to be normal constituents of animal tissue but not present in the perfusing solution (S, P, Mg, Mn, I, Al, Co, Cu, N and Zn); these were negative without exception. Second those present both in the tissue and in the perfusate (Na, Ca and K); in this case comparison had to be made

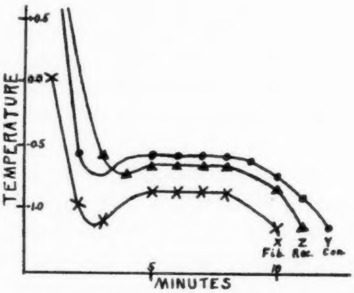

Fig. 1. Cooling curves showing freezing point (plateau) of ventricular muscle.

between the outflowing perfusate before and after the onset of fibrillation. The results for sodium and calcium were likewise negative.

In the case of potassium, however, the results were positive. While the potassium content of the perfusate was unchanged by passage through the normally beating heart, immediately after fibrillation began the outflowing perfusate showed an appreciable increase in the amount of potassium present. The analytical method of Kerr was followed (2). Although it is realized that potassium analyses are beset with pitfalls we believe our methods to be trustworthy since every safeguard against error was taken and more especially since the samples were given blind numbers so that the analyses were made without prejudice. In spite of the uncertainties inherent in the method used this finding is important because of the influence exerted by potassium on the normally beating heart and because potassium may be used to recover normal behavior in a heart thrown into ventricular fibrillation (3).

In nine experiments the average potassium content of the out-flowing

perfusate, in milligrams per cent, before fibrillation was 12.8 and shortly after fibrillation it was 15.0. These values are relatively comparable. The actual amount of potassium in the perfusate varied somewhat in different experiments and the time of collection of the second sample also varied in different experiments. The results, however, were concordant in all experiments and point to a primary upset in the tissue metabolism which is somehow related to the liberation and outward diffusion of potassium ions.

This liberation of potassium would thus appear to be in direct relation to the onset of fibrillation. Samples collected one-half to one minute after fibrillation was established showed the maximum amount of potassium while those taken after fibrillation had continued 3 to 4 minutes often showed a return to the pre-fibrillation value.

This association of readily diffusible potassium with the onset of ventricular fibrillation produced by mild electrical stimulation, however attractive as a working hypothesis, must be definitely qualified. It has been shown that the application of severe electrical stimulation to the heart not only fails to establish ventricular fibrillation but is actually effective in restoring a fibrillating heart to normal functional behavior (4).

In the course of these experiments opportunity was had in two cases to analyse the potassium content of the outflowing perfusate before and after the application of an electrical stimulus of a strength above that which will cause fibrillation. The average values in milligrams per cent were 12.7 and 14.1 respectively. The difference here is less (1.9) than when a fibrillation inducing stimulus was employed (2.2) but because of the limitations of the analytical method and because only two observations were made little emphasis can be placed on the point. Thus with the evidence at hand we can not say that there is a specific relationship between the liberation of potassium by the muscle fibre and the onset of fibrillation.

Modification of microscopic structure. The heart of an anesthetized intact dog is readily thrown into ventricular fibrillation by the passage of a suitable electrical current with resultant death. Post-mortem histological examination of the cardiac muscle under such conditions shows no abnormality. Similarly frog sartorius muscle thrown into a twitching state by passage from Ringer solution to sodium chloride solution is perfectly normal in histological appearance. These points are to be held in mind in connection with the following presentation.

When the perfused and isolated dog heart is thrown into ventricular fibrillation and bits of tissue are examined microscopically the histological picture is that shown in figure 2. Our procedure here was similar to that in obtaining tissue for freezing point determinations, i.e., bits of ventricular muscle were cut off before, during and after fibrillation. They were at once dropped into Zenker's fluid and subsequently examined by Dr. A. R.

Rich to whom we are greatly indebted for this assistance and for the following report:

The fibres of the myocardium of the heart in fibrillation are altered in an extraordinary way which can be best appreciated by an examination of the microphotographs. Briefly, the lesion consists in hyalinization and swelling of the ends of the individual fibres. In these swollen, homogeneously pink-staining areas no striations are visible, but the striations in the remainder of the same fibre are plainly seen, and it is perfectly clear that only a part of the fibre is involved in the hyalinizing process. The nuclei appear quite normal. This curious alteration of the fibres occurs in scattered foci, the muscle fibres between the foci remaining quite normal.

Fig. 2. Shows high and low magnification of ventricular muscle taken from perfused heart in fibrillation.

One is at once reminded of the familiar hyaline degeneration of skeletal muscle which occurs so commonly in the human being, for the appearance of these heart muscle fibres is precisely like that in the hyalinized skeletal muscle fibres. Hyalinization of skeletal muscle is ordinarily regarded as representing an irreversible change in a dead fibre, but if one examines such lesions carefully it is not unusual to see that only a part of an individual fibre is hyalinized while the striations of the rest of the fibre are quite normal. In view of the fact that the present material indicates that rapid recovery from the hyalinized state is possible when it affects only part of the fibre, one is disposed to wonder whether the partial hyalinization of a skeletal muscle fibre may not also represent only a temporary alteration. When one looks at these hyalinized areas in the heart muscle it seems incredible that they could rapidly disappear, with the return of the striations to the area, and perhaps the limitations of the experiment, which prevent one from examining areas from the entire heart during fibrillation and after recovery, may be responsible for the fact that the areas

taken during fibrillation show much more extensive foci of hyalinization than do those taken before fibrillation or after recovery. The only way in which one might obtain more definite information about the possibility of the disappearance of the change on recovery from fibrillation would be to study a much larger series of hearts under the same conditions.

I have never seen a change quite like this in human heart muscle, whether in persons dying with auricular or ventricular fibrillation, nor have I ever seen it in the hearts of animals at autopsy. Hyalinization of dead fibres does, indeed, occur in the human being in various pathological conditions, and focal hyalinization of the type described by German writers as "koernig-scholliger Zerfall" is also occasionally observed in infections; but the present lesion, localized as it is at the ends of the individual fibres, is quite unlike anything that I have ever seen.

It is of interest that the change was completely absent when the dog's heart was thrown into fibrillation within the animal's body and, on the other hand, that an occasional hyalinized fibre was found in the hearts perfused outside the body even before general fibrillation had been induced. This indicates very strongly that the occurrence of the lesion is dependent in part at least upon the abnormal conditions attendant upon the process of artificial perfusion, and this perhaps should place one on guard against applying too readily to the intact heart results which are obtained during perfusion outside the body, for such a profound morphological alteration as this is must surely be associated with definite chemical and metabolic alterations.

The points of immediate interest in this report are: *a*, the exaggeration of the condition in fibrillation, and *b*, the reversible nature of the condition. Thus while we are dealing with a tissue which is not wholly normal since some degree of hyalinization is present in control specimens (a condition not present in hearts similarly fibrillated but with the circulation intact) it exhibits the remarkable capacity to revert to its own normal when the causative disturbance has been counteracted.

Discussion. The foregoing evidence while not conclusive at all points has cumulative force. We have shown, subject to the reservations stated, that the perfused ventricular muscle in passing into the state of fibrillation undergoes a change such that the freezing point of the tissue is lowered. This change is accompanied by a visible alteration in the microscopic structure of the muscle fibre. We have further shown that these changes in molecular aggregation and anatomical configuration are reversible processes. Along with these are the additional facts that of all the inorganic constituents present in the muscle potassium alone undergoes a change by outward diffusion and that momentary increase in the potassium content of the perfusate brings about a complete recovery from fibrillation. Circumstantially, therefore, potassium seems the most probable incriminating factor in relation to ventricular fibrillation.

CONCLUSIONS

When the perfused dog heart is thrown into ventricular fibrillation by electrical stimulation the following changes occur: *a*, the freezing point

of the tissue is lowered; *b*, the microscopic picture of the tissue is changed, and *c*, the potassium content of the out-flowing perfusate is increased. The first two of these changes are reversed by a momentary increase in the potassium content of the perfusate which at the same time has the effect of restoring a normal sequential beat in the heart.

It is therefrom postulated that an unbalance of the readily diffusible potassium ion is associated with the phenomenon of ventricular fibrillation.

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WEIGHT LOSS CHANGES DURING MUSCULAR WORK FOLLOWING FOOD INGESTION

CARL IVER HOVLAND

Institute of Human Relations, Yale University[1]

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It has frequently been suggested that efficiency of performance can be measured quantitatively by using as a criterion the amount of energy consumed in doing a given amount of work. For this purpose oxygen consumption has often been employed as an index of the amount of energy consumption. Numerous studies on the effect of muscular work upon energy transformations have shown marked increases in oxygen consumption, minute volume of the heart, pulse rate and the like. The work of Lovekin (2) shows how these physiological indices may be used in the determination of the efficiency of work. But the difficulties involved in using metabolic indices for this purpose are immediately apparent. There are numerous other factors which affect the metabolism of the organism, and there may be considerable changes taking place due to food, temperature, etc., which have no relevance to efficiency. The plan of the present research is to calculate the ratio between metabolism during work and that during relaxation for determining the changes taking place in the energy used up by the organism, and to use this index rather than the absolute values of metabolism. In this way changes which are brought about by extraneous factors will not affect our results. We are using as our metabolic index the amount of weight lost per unit of time. Our principal interest in the present study is the relative amount of weight lost in doing an equivalent amount of work at various hours following the ingestion of large and small meals.

Method. For the purpose of obtaining accurate readings of weight loss in short intervals of time the Sauter balance was used. The cot upon which the subject rested was mounted directly upon one beam of the balance and counterbalanced on the other side by weights. Sufficient weight was placed upon the scales so that the subject was only slightly heavier than the counterbalance. At the exact moment when the subject and the weights came into equilibrium a stop watch was started. Two additional grams were placed upon the subject's side by the experimenter

[1] Done in the Department of Psychology, Northwestern University. The writer expresses deep appreciation to Prof. G. L. Freeman for facilities and assistance.

and the time required for the subject to lose these two grams was obtained by stopping the watch when equilibrium was again reached. Three such determinations were made while the subject was in a relaxed condition. Three more readings were taken while muscular work was being performed. The task consisted of flexing and relaxing the index finger of the left hand as rapidly as possible. A work adder was mounted on the cot so that the amount of work done was recorded without requiring the subject to change his body position.

Ten normal, healthy subjects were employed. Their age range was from 18 to 34 years, their weight from 132 to 178 pounds, and their height

TABLE 1

Weight loss during relaxation and during finger oscillation following the ingestion of a light lunch

	BEFORE INGESTION	HOURS AFTER FOOD				
		1	2	3	4	5
Loss per hour during relaxation	33.8	36.5	37.1	35.4	34.4	34.2
Loss per hour during work	35.2	39.1	40.9	38.6	41.3	40.4
Ratio of loss during work to loss during relaxation	104	107	110	109	120	118

TABLE 2

Weight loss during relaxation and during finger oscillation following the ingestion of a heavy lunch

	BEFORE INGESTION	HOURS AFTER FOOD				
		1	2	3	4	5
Loss per hour during relaxation	34.5	38.5	40.0	42.4	38.5	36.1
Loss per hour during work	37.3	47.0	45.6	49.1	48.1	43.7
Ratio (in per cent) of loss during work to loss during relaxation	108	122	114	116	125	121

from 64 to 71 inches. At the commencement of the experimental day the subjects were in a basal, post-absorptive condition.

The meals were given at twelve o'clock noon. Weight loss readings were taken hourly, the first one being just before food ingestion. The subjects continued to relax on their cot throughout the experiment. Room temperature was maintained at 70 degrees Fahrenheit. Light, loose clothing was worn by the subjects.

After a day of preliminary orientation to the apparatus and procedure, the subjects were tested for four days. On the first and third day they

were given a light meal, described below, and on the second and fourth day a heavy meal. Half of the subjects were given the light meal first, and half were given the heavy. The light meal consisted of one ham sandwich and one glass of milk; the heavy meal was prepared by tripling the above rations.

Experimental results. Table 1 gives the number of grams lost per hour in the relaxed condition, the number lost during the finger oscillation and the ratio of loss during oscillation to loss during relaxation (in per cent) following the ingestion of the light meal. In table 2 are given the same data for the ten subjects after the ingestion of the heavy meal.

Discussion. It will be observed that the changes in weight loss following the intake of large and small meals during relaxation are identical with those shown in an earlier paper of the writer (1). There is a greater rise following the heavy meal and its peak is later than the rise following the light meal.

The ratios of weight loss during the finger oscillation to the loss during relaxation show that in terms of energy expenditure a light lunch is more favorable to efficient performance than is a heavy meal. Its advantage is most marked in the earlier part of the afternoon. There is a general rise in energy cost, with the maximum increase in weight loss during work over that required during relaxation at four o'clock.

The changes correlate very closely with subjective impressions of the efficiency of performance which were obtained from the subjects. Their statements indicated that they had a feeling of sluggishness following the heavy meal, a fact which is reflected in the greater weight loss required to perform a standard amount of work, and that there was a late afternoon slump, also reflected in greater expenditure at four o'clock. The end spurt at the conclusion of the work is marked, correlating with relief at the end of the day's work.

SUMMARY

Weight loss was recorded during relaxation and during the performance of finger oscillation following the ingestion of large and small meals. Ten subjects were used four days each. Efficiency, as judged by the increment of weight loss during work as compared to the loss during relaxation, appears better after a light lunch, the advantage being greatest in the earlier part of the afternoon, immediately after eating. Maximum inefficiency, from the standpoint of energy expenditure, is found in the late afternoon, about four hours after eating.

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THE RÔLE OF THE HYPOPHYSIS IN EXPERIMENTAL CHRONIC ADRENAL INSUFFICIENCY[1]

ARTHUR GROLLMAN[2] AND W. M. FIROR[3]

From the Department of Pharmacology and Experimental Therapeutics, and the Department of Surgery, The Johns Hopkins University

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It is generally assumed that the adrenal glands and the hypophysis are mutually related in their physiological functions, for extirpation of either of these organs is accompanied by anatomical changes in the other.

Clinically, one observes changes in the pituitary in diseases involving the adrenal cortex and changes in the adrenal in acromegaly, hypophyseal cachexia and other pathological involvements of the hypophysis.

We have observed that animals maintained for some time in a state of chronic adrenal insufficiency assume a clinical picture which is essentially that of hypophyseal cachexia. The physiological deficiencies manifested by such animals are not remedied by treatment with the adrenal cortical hormone but respond to treatment with extracts derived from the pituitary. We must conclude, therefore, that the hypophyseal insufficiency induced by interference with normal adrenal activity is primarily responsible for producing an essential part of the syndrome observed in animals suffering from chronic adrenal insufficiency.

Experimental chronic adrenal insufficiency. Several methods of inducing chronic adrenal insufficiency have been utilized in the present study. The first consisted in treating completely adrenalectomized animals (rats, cats and dogs) for extended periods with a minimal amount of the adrenal cortical hormone sufficient to maintain life but in insufficient dosage to maintain growth in young animals or to maintain normal body temperature, body metabolism, activity of the reproductive organs and general normal activities in adult animals. It is extremely difficult to maintain such a state of insufficiency in young animals for an extended period of time but in matured full-grown animals it may be done with ease.

The second method of inducing a chronic adrenal insufficiency consisted

[1] Studies on the adrenal. IXth communication.

[2] Aided by grants from the Ella Sachs Plotz Fund and the National Research Council for which we wish to express our appreciation.

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in an incomplete adrenalectomy of one-month-old rats. The operation being incomplete, cellular residues remained which eventually gave rise to sufficient gland to maintain life after treatment of these animals for a week to ten days (Grollman and Firor, 1933) with an active preparation of the adrenal cortical hormone.

A third procedure for producing a chronic insufficiency consisted in ligating the blood supply to the adrenals. Although a large percentage of the animals on which this operation was performed succumbed to the effects of an ensuing acute adrenal insufficiency, some survived and developed the symptoms of chronic insufficiency.

The general symptomatology of animals in a state of chronic adrenal insufficiency induced by the methods just described differs from that observed in acute adrenal insufficiency in several important respects. Acute adrenal insufficiency responds readily to treatment with the adrenal cortical hormone, is rapidly fatal in untreated animals, and is accompanied by a rapid loss of weight and other symptoms characteristic of adrenal insufficiency. Animals brought into the state of what we shall describe as a chronic adrenal insufficiency live for long periods of time without treatment, do not respond to adrenal cortical therapy, maintain a constant body weight and are free of many of the symptoms characteristic of acute adrenal insufficiency. Such animals show a slight degree of asthenia, fail to gain weight under a luxus diet, maintain a slightly lowered body temperature, and show a diminished capacity for reproduction. At autopsy evidences of pathological changes are noted chiefly in the degree of inanition and loss of general body fat, marked atrophy of the reproductive system, atrophy of the thyroid gland, and a generalized hyperplasia of the lymphatic system with regenerative enlargement of the thymus. This hyperplasia of the thymus occurs in adult cats and dogs as well as in rabbits and rats, in which it has been frequently observed by previous workers.

The time necessary for inducing this condition of chronic adrenal insufficiency depends chiefly upon the age of the animal and the degree of adrenal insufficiency to which the animal is subjected. In adult rats, cats, and dogs, the condition which we shall describe in the subsequent sections requires several months for its development. Our observations were carried out during the third month following bilateral adrenalectomy, the animals being maintained from the time of operation on an amount of adrenal cortical hormone sufficient to maintain life but insufficient to maintain normal physiological activity. In month-old rats, on the other hand, an acute insufficiency induced by the second method described in the preceding section will give rise to the condition after a period of only a few weeks. In such animals, also, insufficient therapy for as short a period as a week will often induce an irresponsiveness to subsequent adequate ther-

apy which, as we shall see, is due to the development of secondary changes which in turn are responsible for many of the manifestations of chronic adrenal insufficiency.

Growth of rats in chronic adrenal insufficiency. The growth of rats may be stunted by interference with normal hypophyseal or adrenal function. In figure 1 have been reproduced the growth curves of rats, illustrative of

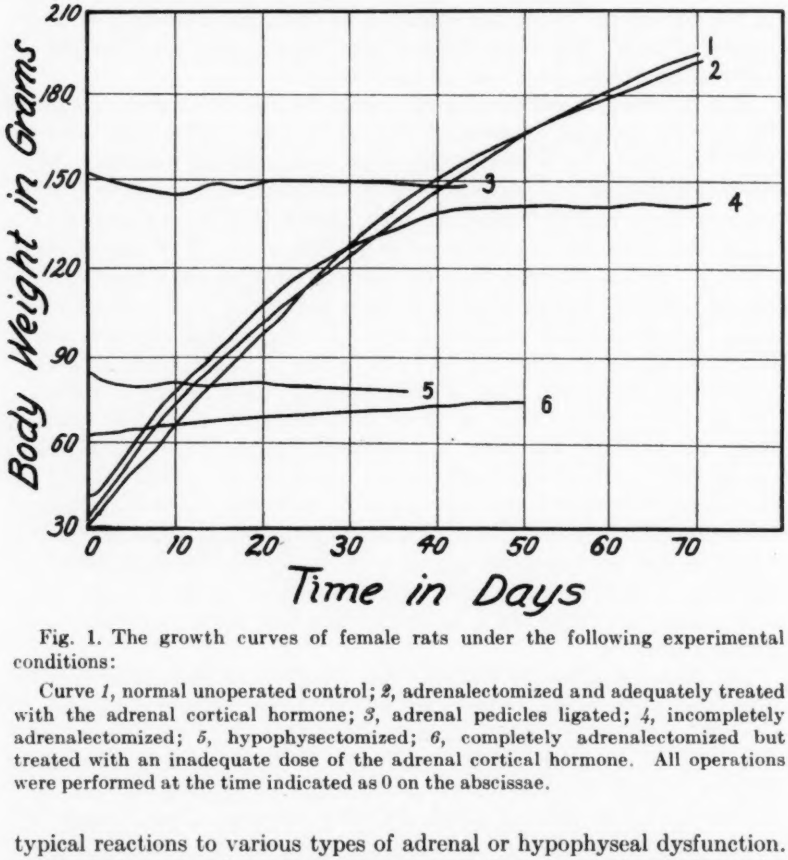

Fig. 1. The growth curves of female rats under the following experimental conditions:

Curve *1*, normal unoperated control; *2*, adrenalectomized and adequately treated with the adrenal cortical hormone; *3*, adrenal pedicles ligated; *4*, incompletely adrenalectomized; *5*, hypophysectomized; *6*, completely adrenalectomized but treated with an inadequate dose of the adrenal cortical hormone. All operations were performed at the time indicated as 0 on the abscissae.

typical reactions to various types of adrenal or hypophyseal dysfunction. Adrenalectomy results in a permanent cessation of growth which can be entirely prevented by adequate replacement therapy with the adrenal cortical hormone (Grollman, Firor and Grollman, 1935). Curve *2* of figure 1 shows the normal growth of such a treated rat as compared with the unoperated litter-mate control illustrated in curve *1*.

Failure to treat an adrenalectomized rat results in death in the course

of a few days to a month depending upon the age and condition of the animal (Jaffe; Pencharz, et alii; Firor and Grollman). However, should the adrenal cortical hormone be administered in small doses one is able to prolong the life of adrenalectomized young animals without supplying sufficient hormone to permit growth. Stunting of growth in this manner is illustrated in curve *6* of figure 1. Incomplete removal of the adrenals, leaving only a minute portion of the glomerulosa of the gland, suffices for regeneration of the gland (Pencharz, Olmsted and Giragossintz) and prolonged survival. In many cases such animals assume their normal adult size and manifest no symptoms of adrenal insufficiency. In some cases, however, one observes that such animals after a preliminary period of growth ultimately cease to grow and maintain a constant weight for long periods of time as illustrated in curve *4* of figure 1. The preliminary normal growth observed in this animal is similar to that noted by Collip, Selye and Thomson in hypophysectomized young rats in which growth does not cease until some time after operation.

Curve *3* illustrates the stunting of growth observed in an animal in which the adrenal pedicles were ligated. Cessation of growth in this animal is similar to that noted in curve *5* which illustrates the cessation of growth in a rat after hypophysectomy.

Effects of adrenal and hypophyseal therapy on growth in rats. In figure 2 are reproduced typical curves which show the effects of administering the adrenal cortical hormone or the growth hormone of the anterior pituitary body to rats whose growth had been stunted by the various procedures cited in the preceding section. The periods of treatment extended for 20 successive days, each period of treatment alternating with periods of an equal length of time during which no treatment was administered. Many animals succumbed before the expiration of the long experimental period cited for the animals in figure 2. However, each part of the experiment, i.e., a 60-day experiment in which the animals were maintained untreated for 20 days, received either adrenal cortical or anterior pituitary growth hormone for 20 days, and finally were observed for 20 more days without treatment, was repeated six times on groups of three rats with the same results as are shown in the more prolonged experiments of figure 2.

Adrenal cortical hormone was administered either in the form of an extract (Grollman and Firor, 1933) injected intraperitoneally daily or in the more satisfactory form of the charcoal-hormone preparation administered orally (Grollman, Firor and Grollman, 1935). To ensure adequate therapy at least 3 rat units of hormone (Grollman and Firor, 1934) were administered daily. The growth hormone of the anterior pituitary was administered intraperitoneally in 1 cc. doses. Part of the extracts were prepared by the method of Putnam, Teel and Benedict as modified by Bugbee, Simmond and Grimes. A satisfactory preparation was also gener-

ously supplied by Dr. J. A. Morrell of E. R. Squibb and Sons, to whom we wish to express our indebtedness.

As shown in figure 2, administration of the adrenal cortical hormone is

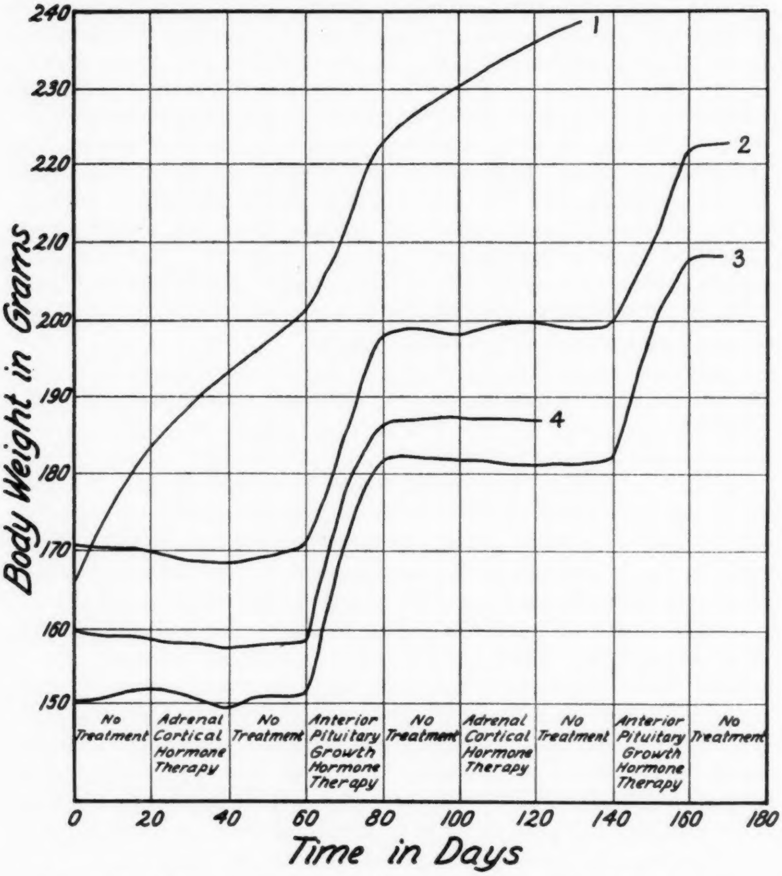

Fig. 2. The effect of adrenal cortical and anterior pituitary growth hormone therapy on the growth-curves of female rats under the following experimental procedures:

Curve *1*, normal unoperated control; *2*, adrenal pedicles ligated; *3*, incompletely adrenalectomized; *4*, hypophysectomized.

The animals were given no treatment during the twenty day periods indicated on the abscissae as 0 to 20, 40 to 60, 80 to 100, 120 to 140 and 160 to 180 days. Each animal received 3 rat units of the adrenal cortical hormone daily during the periods of 20 to 40, and 100 to 120 days. They received 1 cc. of anterior pituitary growth hormone extract daily during the periods of 60 to 80 and 140 to 160 days.

without the least effect on the growth of rats stunted by chronic adrenal insufficiency or by hypophysectomy. On the other hand the administration of extracts of the anterior lobe of the pituitary is accompanied by a remarkable growth approximating or exceeding that observed in normal animals. It may be concluded, therefore, that the cessation of growth in animals maintained for long periods in chronic adrenal insufficiency is not due to lack of the adrenal secretion but is due to pituitary insufficiency induced secondarily by the adrenal insufficiency.

The reproductive system in chronic adrenal insufficiency. The reproductive function of animals in adrenal insufficiency is in abeyance as numerous observers have demonstrated. We have verified this on a large series of rats, cats and dogs maintained for long periods in a state of chronic insufficiency. In the rat, in which, because of the shortness of the oestrual cycle, these observations are best followed, the periods of diestrus may extend for months during chronic adrenal insufficiency. However, in animals in which growth has ceased for several months, spontaneous oestrus may still occur at intervals of several months, compared to 4 to 7 days for normal rats. The animals conceive and may rear a normal litter. About half the animals die during pregnancy or lactation. In such cases there is no evidence of failure of mammary function and the young are normally nourished. Undoubtedly, the strain of pregnancy like other strains upon the organism (excessive heat, cold, drugs such as histamine, etc.) which are innocuous to normal animals can not be borne by animals in chronic insufficiency. In animals surviving the period of lactation, the body weight is found to be the same as before pregnancy. Any growth hormone in the fetuses is apparently not transmitted to the mother, for the stunting of growth continues throughout life. A second pregnancy occurs rarely.

Male animals in chronic adrenal insufficiency show impotence with atrophy of the reproductive system similar to that observed in hypophysectomized animals.

In order to determine whether the adrenal or the hypophysis is responsible for the dysfunction of the reproductive system, hormones derived from these glands were administered to female rats for several months. Repair of the reproductive system did not occur when adrenal cortical hormone was administered in doses of two to three rat units daily. On the other hand, a ready response, similar to that obtained in hypophysectomized animals (Smith), was elicited by injections of extracts of the pituitary, prepared by the method of Fevold, Hisaw and Leonard.

The above observations indicate that the hypophysis is probably responsible for the observed failure of the reproductive system in long continued chronic adrenal insufficiency. The fact that normal reproductive activity can occur in animals whose growth has been permanently stunted

is physiological evidence for the separateness of the growth and reproductive functions of the hypophysis.

The body temperature in chronic adrenal insufficiency. Certain metabolic changes are common to both hypophysectomized animals and those maintained in a state of chronic adrenal insufficiency. Thus, the body temperature is reduced about equally in both cases. In neither hypophysectomized animals nor those maintained in a state of chronic adrenal insufficiency for long periods could the body temperature be elevated to normal by administration of the adrenal cortical hormone. On the other hand, administration of either desiccated thyroid, orally, or the injection of thyroxine[4] or anterior pituitary extracts containing the thyrotropic principal resulted in an elevation of the body temperature to normal. In

TABLE 1

The body temperature of animals in a state of chronic adrenal insufficiency

The data in the table represent averages of daily temperatures obtained during the course of one week. The average deviation of the individual readings from the mean values recorded in the table was less than 0.3°.

NUMBER OF ANIMALS IN SERIES	ANIMAL SPECIES	OPERATIVE PROCEDURE	RECTAL TEMPERATURES		
			Untreated	During thyrotropic hormone therapy	During thyroid medication
			°C.	°C.	°C.
4	Dogs	Normal controls	39.6		
2	Dogs	Hypophysectomized	38.7	39.8	39.5
2	Dogs	Chronic adrenal insufficiency	38.6	39.7	39.8
8	Rats	Normal controls	39.1		
4	Rats	Hypophysectomized	36.5	39.2	38.7
4	Rats	Chronic adrenal insufficiency	36.6	39.3	39.0

table 1 are summarized the results obtained on a series of rats and dogs hypophysectomized or in chronic adrenal insufficiency. The results of table 1 may be interpreted as due to successive changes in the *adrenal-pituitary-thyroid* complex. The primary adrenal insufficiency causes an irreversible injury of the anterior pituitary. This pituitary dysfunction, in turn, results in a thyroid insufficiency which gives rise to the observed symptoms.

The above interpretation of the thyroid deficiency in chronic adrenal insufficiency avoids the paradox otherwise presented by the observations of thyroid hyperactivity in acute adrenal insufficiency (Marine). The latter condition may be considered as a direct effect of adrenal insufficiency

[4] We are indebted to E. R. Squibb and Sons for a generous supply of crystalline thyroxine utilized in this work.

while the results which we have described for chronic insufficiency are due to a secondary pituitary dysfunction.

Anatomical considerations. Histological examination of the anterior lobe of the hypophysis of animals subjected for long periods to adrenal insufficiency revealed changes which may be taken as indicating the anatomical basis for the physiological deficiencies described in the preceding sections.

Dogs maintained on minimal doses of adrenal cortical hormone for periods of 100 days and then allowed to die of adrenal insufficiency revealed changes in the pituitary which resemble those reported in patients dying of Addison's disease (Kraus, 1927). There was an increase in vascularity of the hypophysis, dilatation of the capillaries, and a marked diminution in the number of basophilic cells which, in one dog, had completely disappeared. In the rat, the increased vascularity was less striking than in the dog and there was not as marked a diminution of the basophilic cells. However, the staining of these basophilic cells was very abnormal. As in Addison's disease the changes in the eosinophilic and chromophobic cells were not as striking as the changes observed in the basophilic cells.

Histological examination of the reproductive organs and the thyroids of animals maintained for long periods in chronic adrenal insufficiency demonstrated the same atrophic condition as has been described for hypophysectomized animals (Evans, Smith).

Discussion. It should be emphasized that the observations cited in the present paper were obtained only in animals maintained for a long period in chronic adrenal insufficiency. It apparently requires a prolonged period of mild adrenal insufficiency or a shorter period of an acute insufficiency to induce an irreparable injury to the hypophysis. It is quite possible that other symptoms of chronic adrenal insufficiency than those described here (e.g., the enlargement of the thymus, disturbances in carbohydrate and fat metabolism, etc.) may also be due to secondary hypophyseal dysfunction rather than to the primary adrenal injury. It is significant to note in this connection that the chronic adrenal insufficiency induced by an incomplete adrenalectomy in young rats occurs despite the presence of considerable regenerated adrenal cortical tissue. Since this regeneration occurs rapidly it is logical to suspect extra-adrenal factors as being responsible for the ultimate manifestations which we have described.

There have been attempts recently to attribute the failure of patients suffering from Addison's disease to respond to extracts of the adrenal cortex to involvement of other organs than the adrenals. Although secondary pituitary insufficiency may be expected to occur in Addison's disease (as is borne out by anatomical findings) the chief symptoms are certainly not attributable to hypophyseal dysfunction but resemble those observed in acute experimental adrenal insufficiency. Failure to respond to treat-

ment with the adrenal cortical hormone in these cases may be more logically attributed to inadequate dosage or impotence of the preparations utilized.

The importance of the pituitary for normal adrenal function has been well established (Evans). The present work establishes the importance of the adrenal for maintaining those functions of the hypophysis which regulate normal growth, reproductive activity and thyroid activity. It need not be assumed that the adrenal elaborates a separate hormone necessary for proper hypophyseal activity, for our most highly purified adrenal cortical extracts suffice to maintain in adrenalectomized animals all the physiological functions attributed to the pituitary. It is only when these animals are subjected to an adrenal insufficiency over a sufficiently long period of time that the hypophysis is injured and produces manifestations which are also observed in acute adrenal insufficiency. It may be argued that these manifestations when observed during acute adrenal insufficiency are in reality also due to hypophyseal dysfunction attributable to the adrenal insufficiency. The improbability of this view is shown by the almost immediate cessation in growth of young rats following adrenalectomy, whereas growth does not cease for some weeks after hypophysectomy (Collip, Selye and Thompson). Moreover, we have not been able to induce growth in rats in acute adrenal insufficiency by the injection of potent extracts of pituitary growth hormone.

The present findings explain the apparent failure of some adrenalectomized animals to respond to adrenal cortical therapy after prolonged treatment. This apparent non-responsiveness is due to the use of an incomplete replacement therapy which, as we have seen, by producing a chronic adrenal insufficiency leads to a secondary hypophyseal deficiency. It is unnecessary to attribute the observed findings to the formation of antihormones as suggested by Collip.

SUMMARY

A state of chronic partial adrenal insufficiency was induced in rats, cats and dogs by various methods. The cessation of growth, failure of normal reproductive activity, and subnormal body temperature which ultimately manifested themselves in these animals were not remedied by adrenal cortical hormone therapy. On the other hand, injection of extracts of the hypophysis relieved the observed deficiencies. It is therefore concluded that hypophyseal insufficiency is induced by a chronic adrenal insufficiency and that this secondary hypophyseal dysfunction is responsible for the observed deficiencies.

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THE EFFECT OF INITIAL TENSION ON THE SPONTANEOUS ACTIVITY AND RESPONSES OF THE NON-PREGNANT CAT'S UTERUS

F. A. SIMEONE

From the Laboratories of Physiology in the Harvard Medical School

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Observations on the influence of initial tension on the responses of striated, cardiac and smooth muscles which contract in response to stimulation have shown that there is an optimum tension at which maximal contractions and liberation of energy occur (see Hampel, 1934, for references). The effects of initial tension on the responses of muscles which relax when stimulated have so far not been studied. It was deemed of interest to determine these effects. The non-pregnant cat's uterus, a muscle which relaxes in response to adrenine, was selected for study.

METHOD. Eight cats were used, under dial anesthesia. The body temperature was maintained constant by means of an electric heating pad. The uterus was approached through a midline abdominal incision. One horn was cut and freed after ligation of the ovarian vessels. The ovarian end was attached to a light writing lever and the vaginal end was fixed by a dissecting needle. The horn was enclosed in a glass cylinder to insure adequate moisture and temperature. Denervation of the uterus was obtained by severing the hypogastric nerves.

A weight pan was suspended from the writing lever at a distance of 2 cm. from the fulcrum, while the uterus was attached on the opposite arm at a point 1 cm. from the fulcrum. The weight of the pan was adjusted so that it just lifted the uterine horn to a vertical position under no detectable stretch. This was considered zero tension.

A dose of adrenine (commercial adrenalin) was selected which would insure a practically maximal response. Usually 0.5 cc., 1:50,000, was found suitable for this purpose. The responses to this dose injected intravenously were then recorded with weights increasing from 0 to 14 grams and repeated in the reverse order.

The base line of the records was complicated by the spontaneous rhythmic activity of the muscle. The responses were measured in centimeters on the record, from the mean of the rhythmic excursions for any given tension to the level of maximum relaxation.

RESULTS. *The effect of tension on the spontaneous rhythmic activity.*

Evans (1926) states that stretching smooth muscle renders it more excitable and institutes rhythmic contractions. He reports also that the greater the stretching force the greater the frequency of these contractions. In the present observations the degree of tension had no influence on the frequency of the rhythmic activity. Spontaneous contractions occurred

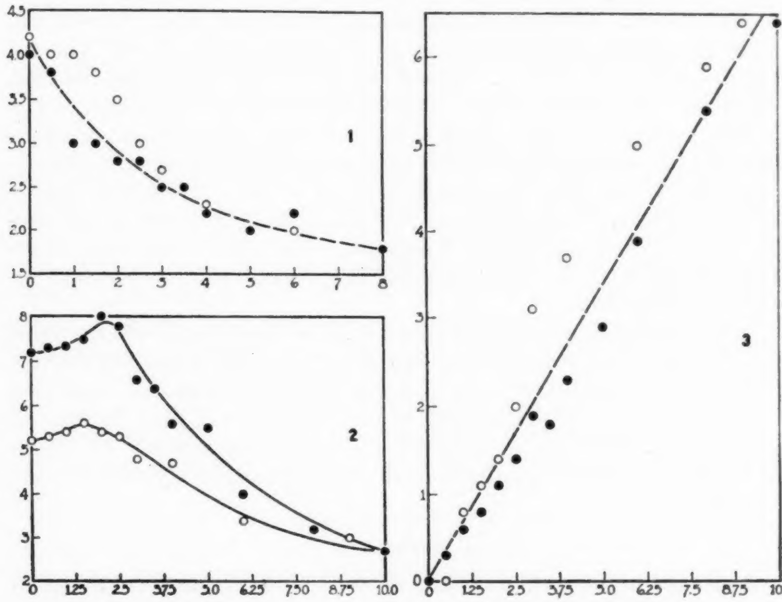

Fig. 1. Effects of tension on the spontaneous rhythmic activity of the uterus. Ordinates: amplitude of the contractions in centimeters in the record; magnification: 6. Abscissae: tension in grams. Dots: increasing tension. Circles: decreasing tension.

Fig. 2. Effects of tension on the responses to a maximal dose of adrenine. Ordinates: relaxation in centimeters in the record; magnification: 6. Abscissae: tension in grams. Dots: increasing tension. Circles: decreasing tension.

Fig. 3. Increments of length as a function of tension. Ordinates: increments in centimeters × 6. Abscissae: tension in grams. Dots: increasing tension. Circles: decreasing tension.

at zero tension and their amplitude diminished with increasing tension. Figure 1 illustrates a typical instance.

The effect of tension on the responses of the non-pregnant uterus to adrenine. Observations on other types of muscle have shown an optimum initial tension for maximal response of the tissue to stimulation. The same relationship holds for the non-pregnant uterus in the cat. A maximal re-

sponse occurs with a load of 1 to 2 grams (effective load of about 1 gram). Figure 2 represents a typical instance. The increment of the responses at the optimum tensions was always slight but obtained consistently.

The influence of tension on the length of the uterus. Within the range of tensions used the mean length of the muscle was found to increase in an approximately lineal ratio with the tension, as shown in figure 3.

DISCUSSION. The purpose of this study was to obtain, if possible, some information regarding the nature of smooth muscle "tone" and the effects of tension thereon. Indeed, Ritchie (1928) has suggested that stretch is the stimulus responsible for "tone" in smooth muscle. The results reported do not support this hypothesis. If stretch caused contraction of smooth muscle we would expect contraction when tension is applied. On the other hand, tension would lengthen the muscle because of its elasticity. Either of these two antagonistic influences might preponderate. We actually find a lengthening (fig. 3), but a concealed stimulation is possible. A similar argument is applicable to the relaxations induced by adrenine.

The influence of tension on the magnitude of the spontaneous rhythmic activity, however, is against the view that "tone" is an effect of stretch. For, with low tension, the stimulus would be applied and the responses (amplitude of the rhythmic activity) should increase, both because of stimulation and because of optimum tension. Experimentally the amplitude of the rhythmic contractions decreases progressively with tension (fig. 1).

SUMMARY

Increasing initial tension causes a decrease in the amplitude of the spontaneous rhythmic contractions of the non-pregnant cat's uterus (fig. 1), while it has no effect on their frequency.

There is an optimum tension for the relaxations elicited by adrenine fig. (2).

The length of the uterus increases in an approximately lineal ratio with the tension (fig. 3).

The bearing of these data on smooth muscle "tone" is discussed.

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ALIMENTARY MOTOR CONDITIONING AND PITCH DISCRIMINATION IN DOGS

SIMON DWORKIN

From the Department of Physiology, McGill University, Montreal, Canada

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In previous experiments the writer (1935) established that the extreme limit of pitch discrimination in the cat is about one tone. This degree of sensitivity is considerably less than that found in the dog by Pavlov and his co-workers (see Andreyev, 1934). The difference in question may either be specific or might depend simply on the different effector responses used in the two cases. The cats had been tested by an alimentary-motor method. On the other hand, most of the workers on dogs have hitherto utilized the salivary reflexes. The main object of the present research was to measure the pitch-discriminating capacity of dogs when tested by the same alimentary-motor method as was applied to the cats. In some experiments, and in order better to compare the two procedures, simultaneous records of salivary and motor responses were taken from the dogs.

The experiments were carried out in a silence-room, under conditions identical with those of the experiments on cats (see Dworkin, 1934). Seven dogs were trained to respond by the motor method to various stimuli, and the limits of pitch discrimination were determined in two.

The mode of training was the same as that used with the cats. During a preliminary period the dogs were taught to obtain food for themselves by raising a hinged lid on the food-container. At a later period feeding was associated with specific stimuli, the latter being delivered, according to circumstances, by a bell, by a buzzer, by a light, by a tactile stimulation method, and by a low frequency pure-tone oscillator. The presentation of a stimulus and also the elicited response were in each case recorded graphically. The unconditioned (food) stimulus was a mixture of meat powder and boiled oatmeal.

Alimentary-motor conditioning. During early training all seven dogs were diffident and easily distracted. They took from one to three weeks to become accustomed to the room and to the conditions of the experiment. Only then were they willing to take food in the experimental chamber and to make attempts to raise the lid of the food-container. To the conditioning stimuli the latent period of some of the early responses was as long as

70 seconds. Once this hesitant or diffident stage was over, conditioned responses were developed easily and were well retained. Interval movement was never frequent, and if initially present, disappeared quickly. The minimum number of trials required to establish firm alimentary-motor responses depended partly on the strength of the stimulus and partly on the character of the animal. In the case of a pure tone of moderate loudness (40 to 50 decibels) conditioned responses could be well developed after 20 tests. A strong bell sometimes became effective after 4 to 5 trials, a tactile stimulus after 9 or 10 trials, while, with white light, responses were never elicited before 25 tests, and often not before 40 or 50 tests.

While the dog proves to be a good subject for alimentary-motor reflexes, it is far more liable than the cat to be affected by accidentally occurring stimuli. Complete inhibition was often produced by the sound of a door slammed near-by, by loud conversation in the experimenter's chamber, or even by some minor change in the arrangement of apparatus in the animal's chamber. On one occasion, after the position of certain battery cells which had hitherto stood in full view of the animals was changed, not merely the conditioned responses but even the eating of food was abolished for a time.

The limits of pitch discrimination. The two dogs used for experiments on pitch discrimination had been previously conditioned to respond to several types of stimulus. For the special tests, the procedure employed was that of "differentiating inhibition" (Pavlov). In the course of its six or seven daily positive tests one negative (not reinforced by food) stimulus was used. As far as possible the loudness of the notes to be differentiated was—in the manner described elsewhere (Dworkin, 1935)—kept equal.

In the case of one dog a note of 2500 cycles per second was chosen as a positive stimulus, one of 3500 cycles as the first negative stimulus. The duration of the negative stimulus was 20 seconds. After 20 trials, occasional discriminations were noted, and after 30 trials the discrimination was complete (zero response to several successive negative stimuli). The negative stimulus was then changed to 3000 cycles per second. This was distinguished from 2500 cycles at the 34th trial. Thereafter 2900 cycles was promptly differentiated. The positive tone was then changed to 2600 cycles. This was differentiated from 2900 cycles at the 40th trial. At the 54th trial, 2700 cycles was distinguished from 2900 cycles, and at the 61st trial, from 2830. The limit of firm discrimination in this dog, attained after 83 trials, proved to lie between 2775 and 2700 cycles per second. This represents an interval of about one-third of a tone. After several more negative tests in attempts to form a closer differentiation, complete inhibition resulted, first to the conditioned, then to the unconditioned stimuli.

The second dog discriminated between 2700 cycles and 2900 cycles in 67 tests, and between 2820 and 2900 cycles in 83 tests. With this animal, too, it proved impossible to develop further differentiation.

These limiting determinations—representing intervals of about one-third of a tone—are quite in agreement with those observed by some of the Russian workers (Zeliony, 1907; Anrep, 1920; Andreyev, 1934). It may therefore be concluded that the dog does really have a greater capacity for pitch discrimination than the cat.

Direct comparison of the salivary and the motor responses.[1] It had thus appeared that, in the matter of pitch discrimination, there is general agreement between the results obtained by the motor method and those obtained by the salivary method of the Russian workers. In order to compare the two methods still further, it was decided to take simultaneous records of the salivary and the motor reactions to specific positive and negative stimuli.

Only after the motor responses to the relevant stimuli were well established did one proceed to record the salivary flow. By means of a metal or glass cup fastened to the surrounding skin saliva was collected from a parotid fistula. To record the flow electrically, a water-filled system and vacuum-tube-operated relay were used. For proper operation of the relay, strong suction must be applied to the liquid in the system. This in turn calls for an air-tight seal between the cup and the skin surface. Whether with Mendeleef's cement, with collodion, or with several other adhesive materials tried, it proved somewhat difficult to obtain a satisfactory seal. When it was desired simply to measure the saliva by displacement of fluid in a horizontal manometer, the Mendeleef cement did, however, afford an adequate joint.

Typical results from some of the successful experiments are shown in figure 1. In general, it may be said that the two methods give equivalent results. Nevertheless, certain differences between the motor and salivary responses were observed. One might best select for comparative comment the latent period, the duration of the response, interval activity and inhibition.

Latent period. Usually both responses occurred simultaneously, though in many instances one preceded the other by some seconds. Records A, B and C of figure 1 show how the time relationship may vary. By way of additional illustration a representative protocol of successive responses to an oscillator-tone stimulus may here be cited.

[1] Some of these experiments were carried out with the kind collaboration of Dr. G. F. Sutherland.

Dog "Whitey" April 26, 1934

TIME	TEST NUMBER	STIMULUS	LATENT PERIOD	
			Motor	Salivary
			seconds	*seconds*
11:41 a.m.	657	2000 cycles	2–3	12–13
11:44 a.m.	658	2000 cycles	2	1.5
11:49 a.m.	659	2000 cycles	2 5	3.5
11:52 a.m.	660	2000 cycles	2	2.2
11:58 a.m.	661	2000 cycles	2.5	2
12:02 p.m.	662	2000 cycles	2.5	4.5
12:05 p.m.	663	2000 cycles	2.5	2.5

Duration of response. Pavlov has divided the salivary reaction into two phases, conditioned and unconditioned. The conditioned saliva is that secreted between the beginning of the stimulus and the presentation of the food. The unconditioned is that which commences when the animal has food in its mouth. These two phases appear separately in the first five records of figure 1.

The recorded motor response is in its nature somewhat different. It sharply exhibits two things, the moment of opening and the moment of closure of the lid. The opening of the lid may be considered as the physiological equivalent of the conditioned flow of saliva. What is noteworthy is that the dog invariably drops the lid before the unconditioned flow of saliva has ceased. In other words, the duration of the recorded motor response is always less than that of the whole salivary flow, which thereafter goes on for a time, to die off gradually.

Interval activity. As already stated, interval motor response rarely occurs in a well trained dog, yet nothing in the way of punishment or physical hindrance is employed to prevent it. When interval movement did occur, it was usually, but not always, accompanied by saliva. Interval saliva was, however, often secreted without motor activity (see fig. 1, F). Furthermore, weak intercurrent stimuli could elicit salivary flow without motor response (fig. 1, F). These results may possibly mean that the threshold for salivary reflex is somewhat lower than for the motor response.

Inhibition. In establishing differentiations, it was noted that the motor responses to the negative stimuli were abolished earlier than the salivary responses. This point is illustrated in figure 1, D and E. A note of 2500 cycles was the negative (delayed) stimulus. At the 17th negative trial two separate, very brief, conditioned motor responses were elicited; the salivary flow began simultaneously with the first motor response and was likewise discontinuous (fig. 1, D). At the 19th negative trial the conditioned motor response was abolished, yet saliva began to flow after a

latent period of 7 seconds, and was, for a time, copious (fig. 1, E). After 30 negative tests the salivary reflex, too, was abolished.

In cases when, after a positive stimulus, the presentation of the food was delayed, the animal dropped the lid to pick it up again once or twice. A precisely parallel discontinuity was often observed in the salivary secretion.

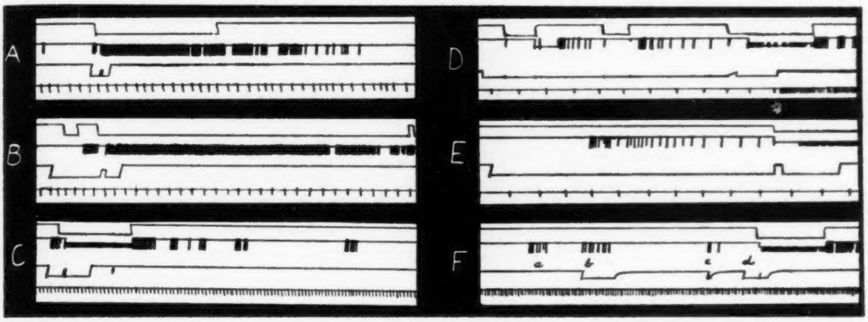

Fig. 1. Six examples of simultaneous records of motor and salivary responses. Read from left to right.

In each case, the top line represents the motor response (lifting and dropping of the lid). The second line represents the salivary flow, in vertical lines. The third line represents the stimulus, while the presentation of the food is indicated by the break in this record. The bottom line represents time in two-second intervals. The kymograph speed was not the same in all experiments.

In record A the motor and salivary responses begin simultaneously; in record B the motor precedes the salivary by 4 seconds, and in record C the salivary precedes the motor by 3 seconds. Whatever the latent period, the total duration of the salivary response always exceeds that of the motor response.

Records D and E show the effect of inhibition (in this case delay) upon the two reflexes. In D the motor response is brief and discontinuous, while a moderate amount of conditioned saliva is secreted. In E the motor response is abolished, while conditioned saliva is still elicited.

In record F interval saliva is shown at *a*, without motor response. At *b* and again at *c* a weak buzzer was sounded. Saliva was secreted, but the lid was not opened. At *d* the regular stimulus was turned on and elicited both responses promptly.

CONCLUSIONS

1. The dog is a good subject for alimentary-motor conditioned reflexes. In the case of this animal, however, special precautions must be taken to avoid the inhibitory effects of extraneous stimuli.

2. The capacity for pitch discrimination of pure tones in the dog is about one-third of a tone as against one whole tone in the cat. This indicates the possession of a superior acoustic analyser by the dog.

3. Simultaneous records of motor and salivary responses to specific positive and negative stimuli show similar end-results. The responses may differ, however, in respect of latent period, duration, interval activity and inhibition.

For financial assistance in this work, the author wishes to acknowledge his indebtedness to the Research Bureau of the American Otological Society.

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ELECTRICAL STIMULATION OF THE INTERIOR OF THE CEREBELLUM IN THE MONKEY[1]

H. W. MAGOUN, W. K. HARE AND S. W. RANSON

From the Institute of Neurology, Northwestern University Medical School

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Investigations of the cerebellar nuclei and the white matter adjacent to them have been very few in number as compared with many studies of cerebellar function based upon gross extirpation of this organ or upon destruction or stimulation of its cortex. It is well known, however, that the nuclei constitute at least the major source of all efferent cerebellar connections, and the small amount of attention which they have received is by no means commensurate with their importance in cerebellar activity. The difficulty of approaching these nuclei, situated as they are within the interior of the cerebellum, has doubtless been the primary obstacle to their study, but this difficulty has been overcome by several investigators.

With the immediate object of investigating the reactivity of the cerebellar nuclei to electrical stimulation, Horsley and Clarke (1) devised their stereotaxic instrument for accurately orienting an electrode within the interior of the brain. Unfortunately their study of the cerebellar nuclei was never completed, and we have only brief and incidental mention of their results (1, 2). At Horsley's invitation this work was continued by Sachs and Fincher (3) but only a preliminary report was made, in which again but brief mention of the results appeared. Some observations of the effect of electrical stimulation of the medial cerebellar nuclei, with the aid of the Horsley-Clarke instrument, have been reported by Mussen (4). In the work of Miller and Laughton (5, 6) on the decerebrate animal, the cerebellar nuclei were electrically stimulated after their exposure by ablating the over-lying cerebellar parts.

In the present investigation, the Horsley-Clarke stereotaxic instrument has been employed in the electrical stimulation of the cerebellar nuclei and the territory adjacent to them in the rhesus monkey (Macaca mulatta).

METHOD. Stimulation of the cerebellum was performed under light nembutal anesthesia (10–19 mgm. per kgm. of body weight) plus supplemental ether added when necessary by means of a tracheal cannula and ether bottle. The animal was suspended untied in a hammock with the limbs hanging free and the head in the freely swinging stereotaxic instru-

[1] Aided by a grant from the Rockefeller Foundation.

ment supported from above. In some of the experiments, the body was supported from above by strings under the supraspinous ligament at the shoulder and pelvis. The technique of electrical stimulation used has been described in detail by Ranson (7), and need be only briefly referred to here.

Using the Horsley-Clarke stereotaxic instrument and a bipolar needle-like electrode, less than 1 mm. in diameter, with the tips of the two wires separated by a distance of 1 mm. along the axis of the electrode, the interior of the cerebellum was systematically explored by electrically stimulating in orderly succession every cubic millimeter of its substance. The current was supplied by a single dry cell registering 1.5 amp. attached to a Harvard inductorium, the secondary coil of which was set at 9 cm.

In eight monkeys stimulation was performed along punctures in a vertical plane, the electrode being inserted through the overlying cerebral hemisphere, after removal of a part of the calvarium and dura, and retraction of the superior sagittal sinus. In four of these animals, stimulation was begun on the left side and extended to the midline and onto the right side. In the other four, stimulation was begun to the right of the midline and extended onto the left side. In two cases the exposure was made through the occipital bone above the foramen magnum, and stimulation of the left side was performed along punctures in a horizontal plane, the electrode being introduced through the caudal, rather than the dorsal, surface of the cerebellum. In every case, stimulation was begun in the caudal portion of the cerebellum and continued rostrally through the cerebellar nuclei to the cerebellar peduncles. The intact efferent cerebellar pathways, therefore, always lay ahead of every point of stimulation.

Subsequent microscopic examination of serial sections of the explored area enabled the localization of each point stimulated. A correlation between the location of these points and the responses obtained from their stimulation, as represented by appropriately situated symbols on a series of eight transverse sections at millimeter intervals through the interior of the cerebellum, gave a composite and graphic picture of the results obtained.

Results. It is interesting to note that in the order of frequency of response to cerebellar stimulation, the parts of the body have been represented in this series of experiments as follows: eyes, ipsilateral forelimb, head, contralateral forelimb, hind limbs, trunk and tail. In our opinion no particular significance should be attached to this sequence, beyond the suggestion that the anterior part of the body has a greater representation than the posterior.

The responses of the eyes, head, trunk and tail have consisted of deviations from the longitudinal axis of the body and may be grouped together as reactions of the eyes, head and axial musculature. The responses of

the limbs form a second group manifest as reactions of the musculature of the extremities. For convenience in presenting the results, the responses of the two groups may be taken up separately, but reactions of both groups have occurred together from stimulation of certain regions of the cerebellum.

The responses obtained from cerebellar stimulation have been definitely postural reactions, as distinct from the quick reactions produced by stimulation of the cortico-spinal system or peripheral nerves, and from the usually phasic responses obtained through reflex activation of the motor system. Such cerebellar responses take a number of forms. They may consist of a slow movement during stimulation to a posture which is maintained throughout stimulation. On the other hand, an inhibition or relaxation of a previously existing muscular contraction often occurs during stimulation. After stimulation, there follows either a sudden rebound to a posture usually representing the opposite of that produced by stimulation, or a slow assumption of an attitude, usually after repeated stimulation, which may persist as a postural background throughout the experiment.

Eyes, head and axial musculature. The reactions of the eyes, head and axial musculature to electrical stimulation varied with the region stimulated and consisted: first, of a marked conjugate deviation of the eyes and a slight turning of the head (not always present) to the side stimulated, or secondly, of a movement of the eyes and usually the head to a position of forward gaze, each from a position of contralateral deviation existing as a posture slowly assumed during the course of the experiment. In every case these reactions of the first and second type were confined to the eyes and head. Responses of these types have been very widespread throughout the interior of the cerebellum, being obtained from the stimulation of points within the extent of all of the cerebellar nuclei, and the white matter adjacent to them.

Reactions of a third type have concerned not only the eyes and head, but in some instances the trunk and tail also. In the latter case, they represent responses of the entire length of the body axis. These reactions of the third type were made up of two phases, the first occurring during the period of stimulation, and the second occurring as a rebound at the end of stimulation. During the period of stimulation, the eyes and head moved to a position of forward gaze or deviated to the side of stimulation, as before. Immediately following the cessation of the stimulus, the eyes and head briskly and actively deviated to the contralateral side.

Such responses of the head are shown in some of the photographs of figures 1 and 2. In figure 1, A and B, the head was turned slightly to the left during stimulation of the left side of the cerebellum (A), and at the end of stimulation exhibited a rebound deviation to the right (B). In figure

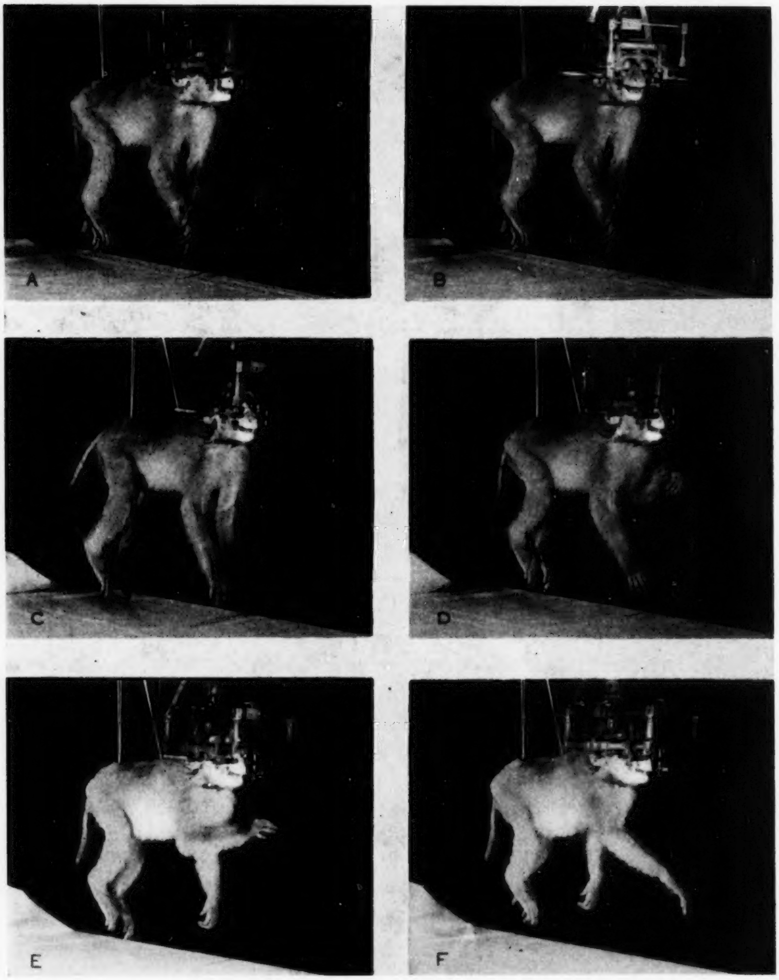

Fig. 1 is made up of three pairs of photographs, each pair showing the responses to stimulation of a single point within the interior of the cerebellum. In each case, the photograph on the left shows the phase of the reaction occurring during stimulation, the photograph on the right the rebound after stimulation. Photographs A and B show a response of the head, stimulation being on the left side of the cerebellum. Photographs C and D show a response of the ipsilateral forelimb, stimulation being on the left side of the cerebellum. Photographs E and F show a response of the ipsilateral forelimb, stimulation being on the right side of the cerebellum. The animals were under nembutal anesthesia and did not experience pain.

2, E and F, the head was turned to the right during stimulation of the right side of the cerebellum (E), and exhibited a rebound deviation to the left at the end of stimulation (F). In figure 2, C and D, the head took a position of forward gaze during stimulation of the right side of the cerebellum (C), and exhibited a rebound deviation to the left at the end of stimulation (D). In the photographs movements of the head can be gauged best by noting changes in the long axis of the stereotaxic instrument.

Responses of the trunk and tail, when present, occurred in two phases. The first phase appeared during stimulation and consisted either of a relaxation of a preëxisting concavity of the body axis to the opposite side, or of the production of a concavity of the trunk and deviation of the tail to the side of cerebellar stimulus. The second phase appeared as a rebound at the end of stimulation and consisted of the production of a concavity of the trunk and a deviation of the tail to the side opposite cerebellar stimulus.

Responses of the body axis do not stand out clearly in the accompanying photographs, but were present in each of the responses shown in figure 2. In figure 2, A and B, the body axis was straightened during stimulation of the left side of the cerebellum (A), and exhibited a rebound concavity to the right at the end of stimulation (B). In figure 2, C and D, the body axis was straightened during stimulation of the right side of the cerebellum (C), and exhibited a rebound concavity to the left at the end of stimulation (D). In figure 2, E and F, the body axis exhibited a concavity to the right during stimulation of the right side of the cerebellum (E), and a rebound concavity to the left at the end of stimulation (F).

These responses of the third type were obtained only from the medial part of the interior of the cerebellum, i.e., from the stimulation of points in the white matter bordering on the roof nuclei, and in some instances also from points on the medial edge of the emboliform nucleus.

Limbs. Postural reactions of the limbs have been obtained both as responses during stimulation and as rebound contractions at the end of stimulation. Two groups of such responses may be differentiated.

Group I of the limb responses includes a number of reactions, usually slight in excursion and confined to parts of the ipsilateral forelimb, or rarely to the ipsilateral hind paw. These responses consisted of two phases and usually made their appearance as a brisk, short-lasting assumption of posture at the end of the period of stimulation. A second stimulation, immediately following, relaxed this posture, but at the conclusion of the stimulus, the attitude was once more assumed. Because of its appearance at the end of stimulation, the assumption of posture appeared to be of the nature of a rebound contraction following inhibition. As a common variation, an attitude was often assumed as a result of a contraction of one muscle group during the period of stimulation, while a brisk rebound con-

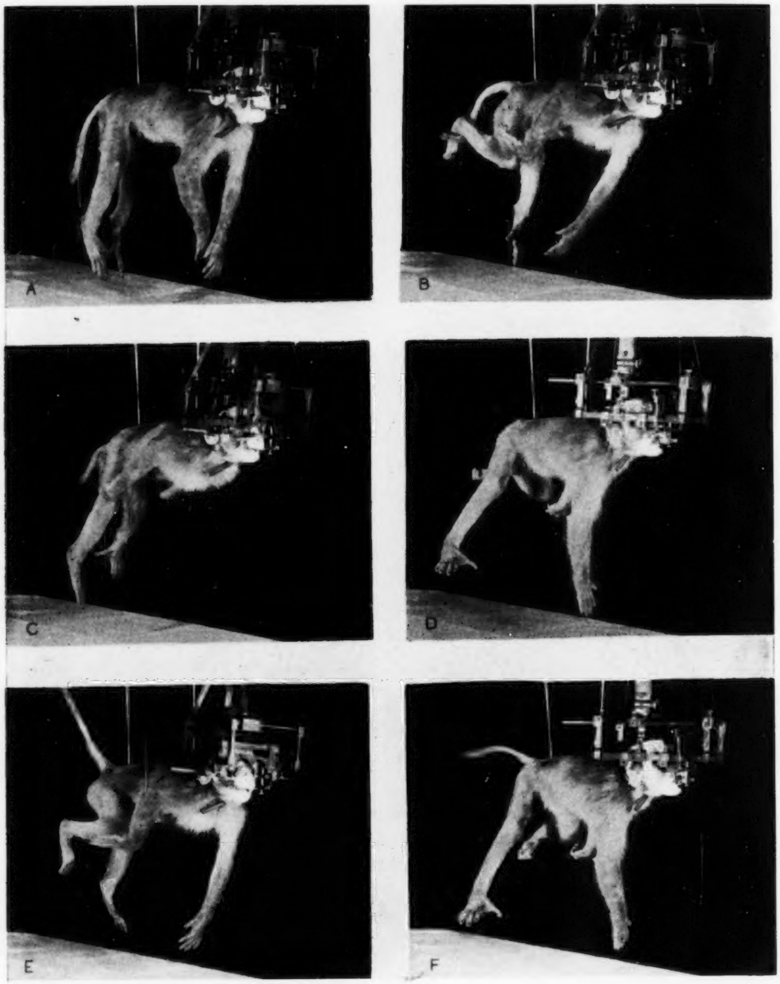

Fig. 2 is made up of three pairs of photographs, each pair showing the response to stimulation of a single point within the interior of the cerebellum. In each case, the photograph on the left shows the phase of the reaction occurring during stimulation, the photograph on the right the rebound after stimulation. Photographs A and B show responses of the limbs and body axis, stimulation being on the left side of the cerebellum. Photographs C and D show responses of the limbs and body axis, stimulation being on the right side of the cerebellum close to the midline. Photographs E and F show responses of the limbs and body axis, stimulation being on the right side of the cerebellum. The animals were under nembutal anesthesia and did not experience pain.

traction of the respective antagonistic muscles at the end of stimulus reversed the attitude in the part concerned. In none of these cases was there a rebound rigidity or resistance to passive manipulation.

Responses of this nature consisted: *a*, of a flexion or relaxation from extension of the forearm, hand, or fingers during the period of stimulation, followed at the end of stimulation by a rebound extension of these parts of the limb; *b*, of a relaxation from flexion during stimulation followed by a rebound flexion of these parts at the end of stimulus; *c*, of a relaxation from pronation or of an active supination of the forearm and hand during the period of stimulation, followed by a rebound pronation at the end of stimulus; *d*, of a relaxation from abduction or an active adduction of the arm during the period of stimulation, followed at the end of stimulus by a rebound abduction. These responses appeared of a diverse nature from the point of view of the muscles and joints involved, but they could be associated in that they were all concerned with postures of parts of the ipsilateral anterior extremity.

Photographs of two reactions belonging to this group are shown in figure 1. In figure 1, C and D, a response is shown of the left forelimb, stimulation being on the left side of the cerebellum. During stimulation the left forelimb was relaxed (C), while at the end of stimulation it exhibited a rebound flexion of the elbow and wrist (D). In figure 1, E and F, a response is shown of the right forelimb, stimulation being on the right side of the cerebellum. During stimulation the right forelimb was flexed at the elbow and slightly supinated (E), while at the end of stimulation it exhibited a rebound extension of the elbow and some pronation of the forearm (F).

Responses of group I were obtained from the buried cerebellar cortex of the anterior lobe both caudal to and overlying the fastigial, globose, and emboliform nuclei. The reactive points converged laterally in the underlying white matter, so that many responses were obtained from the stimulation of points in the region occupied by the emboliform and globose nuclei, while no responses of this nature were obtained from the dentate or fastigial nuclei.

Group II consisted of responses of all four limbs. These reactions were similar to those just described in that the reaction occurred in two phases, and was usually first seen as a rebound assumption of posture at the end of the period of stimulation. A second stimulus relaxed this posture, which was briskly assumed once more as the stimulus was concluded. While a number of variations were observed in the postures involved in the responses of this group, very commonly the reactions consisted of a relaxation of the limbs during stimulation, followed, at the end of stimulus, by a brisk and gross rebound extension of the limbs on the ipsilateral side and a flexion of those on the contralateral side. The rebound was usually

greater in the forelimbs than in the hind and was often most marked in the ipsilateral forelimb. In the marked responses of this nature, the rebound posture was maintained for a period of minutes, as a strong rigidity and resistance to passive manipulation, chiefly of the forelimbs and greatest in the ipsilateral forelimb. In a number of cases such rebound postures were timed and were still undiminished at the end of five minutes. The cycle of inhibition and rebound could be continued indefinitely with repeated stimulation, the responses becoming augmented on repetition.

In some cases, instead of a relaxation of a preëxisting posture during stimulation, an actual contraction occurred in the muscle groups antagonistic to those contracting in the rebound at the end of stimulation. That is, during stimulation there was a flexion of the ipsilateral limbs and an extension of the contralateral limbs, while after stimulation there followed, as before, a rebound extension of the ipsilateral limbs and a flexion of the contralateral limbs. In reactions obtained from stimuli close to the midline, the limbs of the two sides often assumed similar rather than contrary postures, either during or after stimulation.

In whatever form they were manifest the reactions of group II appeared to be concerned not so much with the attitudes seen in small movements of specific parts of a single anterior extremity, as with those involved in the gross postures of all four limbs.

Photographs of reactions belonging to this group are shown in figure 2. In figure 2, A and B, a response is shown to stimulation on the left side of the cerebellum. During stimulation there was a relaxation of all limbs (A), which was followed at the end of stimulation by a rebound extension of the left limbs and a rebound flexion of the right limbs (B). This rebound posture (B) was timed, and at the end of five minutes showed no appreciable relaxation.

Figure 2, C and D, shows a response to stimulation of the right side of the cerebellum, close to the midline. During stimulation both forelimbs were retracted and flexed (C). At the end of stimulation there was a rebound extension of the right limbs and a rebound flexion of the left limbs (D).

Figure 2, E and F, shows a response to stimulation of the right side of the cerebellum. During stimulation the right limbs were flexed and the left limbs were extended (E). At the end of stimulation the right limbs exhibited a rebound extension and the left limbs a rebound flexion (F). The photograph of the rebound posture (F) was taken two minutes after the cessation of the stimulus. A comparison of the rebound postures shown in figure 2, D and F, with that obtained from the opposite side of the cerebellum (fig. 2, B) will demonstrate the striking reversal of response encountered in passing from one side of the midline to the other, reactions of contrary sides being mirror images of one another. A similar reversal

on crossing the midline is seen in the movements of the head. During stimulation of appropriate points on the left side, the head turns to the left and after stimulation rebounds to the right (fig. 1, A and B). Conversely, stimulation on the right side produces a turning of the head to the right during stimulation followed by rebound to the left (fig. 2, E and F).

Reactions of group II were obtained from the buried cerebellar cortex of the anterior lobe, both caudal to and overlying the globose and fastigial nuclei. The reactive points appeared to converge slightly medially in the underlying white matter, so that many responses were obtained from the stimulation of points in the vicinity of and within the substance of the fastigial nucleus, while at least the caudal part of the globose nucleus appeared definitely lateralward of the responsive region. No responses of this nature were obtained from the emboliform or dentate nuclei.

The reactions which have been reported above, from stimulation of the interior of the cerebellum, disappeared as the electrode emerged from the lower surface of the cerebellum into the fourth ventricle. Stimulation of points in the underlying brain stem never has yielded responses of the types described above, but chiefly contractions of isolated groups of muscles supplied by the fifth, sixth, seventh or eleventh cranial nerves.

Discussion. The results just reported indicate the extent to which this method of electrical stimulation may be employed in an investigation of the interior of the cerebellum. The technique does not appear to be applicable to a study of all parts of this region. For example, stimulation of points within the substance of the dentate nucleus, under the conditions of these experiments, has yielded no consistent response specific to this nucleus. There are, however, two regions of the interior of the cerebellum which, in these experiments, have been regularly associated with a consistent type of reaction pattern.

The first of these is the region of the emboliform and globose nuclei and the neighboring white matter, which appears to be closely associated with the limb responses of group I, concerned with small and short-lasting postures of parts of the ipsilateral anterior extremity. The second is the region of the fastigial nucleus and the neighboring white matter, which appears to be closely associated with the limb responses of group II, concerned with gross and long-lasting postures of all four limbs, and probably also with the eye, head, and trunk responses of type III, concerned with lateral deviations from the longitudinal body axis.

The biphasic nature of these responses, consisting usually of an inhibition during stimulation and a rebound contraction after stimulation, suggests their relationship to similar responses obtained by a series of workers from stimulation of the cerebellar cortex in the decerebrate preparation. Reference has been made to a number of these studies in the most recent

report on this subject, that by Denny-Brown, Eccles, and Liddell (8). There are many points of similarity between the results obtained by these authors and our observations on the limb responses of group II, which in this series of experiments have been followed from the buried cortex of the anterior lobe through the underlying white matter to the region of the fastigial nucleus.

This similarity suggests that we are dealing here with one and the same reaction, obtained by Denny-Brown, Eccles, and Liddell from stimulation of the surface of the cerebellar cortex, and obtained in the present investigation from stimulation of the underlying cortex and white matter, and region of the fastigial nucleus. If this be true, the presence or absence of a decerebrate rigidity in the animal would appear to be an indifferent factor, possibly serving only to quantitatively augment the normal reaction of this cerebellar system to the point at which it might be elicited by surface stimulation of the cerebellar cortex.

This suggestion receives support from the fact that the results of the present study are in agreement, in some general features, with the observations of Miller and Laughton (5, 6) obtained from stimulation of the cerebellar nuclei in the decerebrate cat.

SUMMARY

Electrical stimulation of the interior of the cerebellum in the monkey, employing the Horsley-Clarke stereotaxic instrument, has yielded responses of the eyes, head, and axial musculature, and responses of the limbs.

With the possible exception of some reactions of conjugate deviation of the eyes and head to the side of stimulation, these responses have been biphasic in nature, and have consisted of one effect during the period of stimulation, followed by a second effect occurring as a rebound at the end of stimulation.

Such responses have consisted either of an inhibition of a muscle group during stimulation followed by a rebound contraction of this muscle group at the end of stimulus, or of a contraction of one muscle group during stimulation followed at the end of stimulus by a rebound contraction of its antagonist.

Reactions of this nature involving small and short-lasting postures of parts of the ipsilateral anterior extremity have been traced from the buried cortex of the anterior lobe through the underlying white matter to the region of the emboliform and globose nuclei.

Other reactions of this nature involving pronounced and long-lasting postures of all four limbs, the rebound contractions usually persisting for a period of minutes, have been traced from the buried cortex of the anterior lobe through the underlying white matter to the region of the fastigial nucleus. Similar responses of the eyes, head, and axial musculature, in-

volving lateral deviations from the longitudinal body axis, have been obtained from the white matter in the vicinity of the fastigial nucleus.

Somewhat comparable reactions have been observed from cerebellar stimulation in the decerebrate preparation by a series of workers. The suggestion is made that such reactions simply represent normal cerebellar responses, the presence of a decerebrate rigidity being an incidental factor.

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THE QUANTITATIVE DETERMINATION OF URINARY OESTRIN

GEORGE VAN S. SMITH AND O. WATKINS SMITH

From the Fearing Research Laboratory, Free Hospital for Women, Brookline, Mass.
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In analyzing pregnancy urines for oestrin so much of the hormone is present that extraction is not necessary. We have therefore assumed that the results upon slightly acidified unextracted specimens from this source were as quantitatively accurate as could be expected in any determinations in which a biological means of assay must be used. In the case of urines from non-pregnant individuals, however, with which concentration is called for, the striking inconsistency of the results of the different recognized methods of extraction has convinced us of their quantitative inadequacy. We therefore set out, by experiments on assayed pregnancy urines, to test a number of solvents with the purpose of finding some method that would give complete recovery of urinary oestrin. The outcome of these experiments has forced us to the conclusion that all of our previous so-called quantitative values on both pregnant and non-pregnant individuals represent a variable and in most instances only a small part of the oestrin that we have since been able to obtain. Moreover, they are not comparable from a quantitative standpoint and are significant only in that a large number of analyses may have roughly indicated certain general trends.

In all of the experiments to be reported the urine specimens were acidified when received, if necessary, with dilute hydrochloric acid to a pH between 7.0 and 4.0, i.e., acid to litmus but not to congo red paper. Most of the specimens were at this pH when received. No preservative was added but the urines were kept cold and the pH always checked before assay. All extracts were taken up in olive oil unless otherwise specified, since this medium has been found to give slightly higher values than normal saline solution. The technique of assay has been that of Kahnt and Doisy, with rats which had been previously standardized (5). Whenever possible, at least 10 rats have been used in determining the minimum dose, but with the few urines of non-pregnant women which contained less than 5 rat units per 24-hour amount, it was necessary to depend for the result on 3 animals. Twenty-four-hour urines were always collected and the amount of oestrin expressed in terms of rat units per 24-hour volume.

Four different solvents were first tested upon 8 urine specimens from pregnant women and the results compared with assays of the unextracted material. Chloroform, either by continuous extraction according to Frank (1) or by repeated reflux extraction, yielded 13 to 25 per cent as much oestrin. By olive oil extraction according to Doisy et al. (2) 35 to 42 per cent was recovered. Ethyl acetate (3) gave 30 to 60 per cent and continuous 24-hour extraction with benzene[1] in the apparatus shown in figure 1 recovered 72 to 96 per cent of the oestrin found in the particular 8 specimens before extraction. The percentage yields for each of these solvents varied over such wide limits as to make it evident that no one of them would produce even comparable figures. Moreover, when 12 additional pregnancy urines were extracted by the benzene method, the extracts of 7 of them contained up to *5 times as much* as was demonstrable by testing the straight urine. Apparently our original assumption, that assays on unextracted urines could be used as a gauge of the amount of oestrin actually present, was erroneous. Some oestrin must be bound in certain specimens by other urinary constituents and is at least partially freed by the process of extraction. But we could not conclude that benzene freed and recovered all the oestrin present, since in the other 5 instances less was in the extract than in the urine. Neither decomposition nor changes in pH could be found accountable for the inconsistent results. It was apparent that, in order to get true or even comparable values, some method of "freeing" all the hormone before assay or extraction would be necessary.

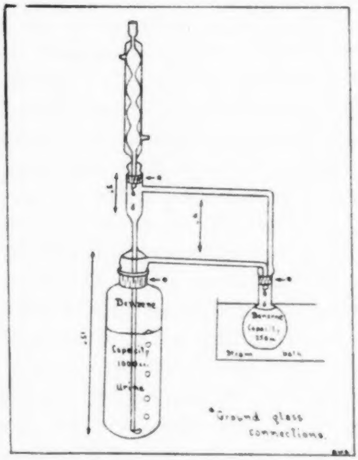

Fig. 1. Apparatus for continuous extraction of urinary oestrin with benzene.

The boiling of the urine of pregnant mares with concentrated hydrochloric acid yields values about 3 times as high as those given by the untreated urine (6). Zondek (8) claims that no preliminary acid treatment is required for the complete removal of oestrin from human pregnancy urines by extraction, but others (2, 9, 10) have found that the oestrin

[1] Merck's benzene (benzol)—reagent. Siebke (4) employs benzene and recommends reflux extraction. In the only instance in which we tried his method we obtained no greater yield than with 24-hour continuous extraction.

obtainable from this source may be augmented by preliminary acid treatment. Since Lipschutz and Poch (6) state that the greatest increase is produced by boiling for 5 minutes with 10 to 20 per cent by volume of concentrated hydrochloric acid, we adopted their procedure. To each 100 cc. of urine, therefore, 15 cc. of concentrated hydrochloric acid were added and the mixture boiled vigorously for 5 to 10 minutes. In the case of ethyl acetate it was necessary to neutralize the urine again to a pH between 7 and 4 before extraction, since otherwise acetic acid is formed. This neutralization does not cause any demonstrable loss of the increased potency *provided the extraction is carried out immediately*. The fact that neutralization is not called for before extracting with benzene further recommends this solvent.

Table 1 presents figures for the comparative yields from untreated and acid treated urines of pregnant women. For the sake of simplicity we shall call the yield from untreated slightly acid urines "free oestrin" and that from urines boiled with hydrochloric acid "total oestrin." The term "total" may be misleading. Although, as will be shown below, no other procedures that we have tried have yielded more oestrin than has 5- to 10-minute boiling with 15 per cent hydrochloric acid, it is possible that some other process might recover or produce even more of the hormone. An increase in the amount of oestrin in all of these specimens followed boiling with hydrochloric acid. The ratios between unextracted "total" and unextracted "free" oestrin, however, vary from 1.5 to 1 (urine no. 7 (b)) to 40 to 1 (urine no. 5(c)), indicating that the "free" oestrin is probably dependent upon variations in the urinary constituents which cause the hormone to be partially inactivated or bound. The figures for "total" oestrin, on the other hand, are as quantitatively satisfactory as could be expected; viz., the amounts in the saline benzene extracts of treated urines check very well with the amounts found in unextracted specimens after boiling with hydrochloric acid, proving complete recovery by benzene. The olive oil benzene extracts rendered the values slightly higher in some instances, due probably to slower and more uniform absorption. Ethyl acetate extraction is not as complete as benzene but, when applied to urines that have been boiled with acid, probably gives roughly comparable figures.

It still remained to be confirmed that 5- to 10-minute boiling with 15 per cent acid gave as complete a yield as was possible by acid treatment. Eleven samples of a pregnancy urine were treated with different amounts of hydrochloric acid and heated for varied periods. The procedures and results are summarized in table 2. It was concluded that in order to obtain the greatest oestrogenic potency without any destruction, urine should be boiled for 5 to 60 minutes after adding 15 to 30 per cent by volume of

TABLE 1

Demonstrating the augmented oestrogenic potency of human pregnancy urines after boiling with acid, and the quantitative recovery of "total" oestrin by benzene extraction

SOURCE OF SPECIMEN	OESTRIN RECOVERY—RAT UNITS IN 24° VOLUME					
	Untreated (pH of 7.0 to 4.0) "Free"			Boiled with 15 per cent HCl—"Total"		
	Unextracted	Extracted with ethyl acetate	Extracted with benzene	Unextracted*	Extracted with ethyl acetate	Extracted with benzene
1. Mrs. A. P. 7 mos. pregnant	5100	3100			15000	
2. Mrs. P. 7 mos. pregnant	4000	1800	2800		14000	16000
3. Mrs. L. 6 mos. pregnant	1200	620	950		3300	5000
4. Mrs. C. 6 mos. pregnant	2500	770	2400		20000	30000
5. D. L. (a) 6 mos. pregnant	3000		8600	15000		20000
			6000†			15000†
(b) 7 mos. pregnant	3100		15000	42000		44000
(c) 8½ mos. pregnant	2900			115000		
6. L. L. (a) 6 mos. pregnant	1500		750	3000		3700
						3200†
(b) 7 mos. pregnant	970			24000		
(c) 8½ mos. pregnant	42000			95000		
7. M. M. (a) 6 mos. pregnant	600		470	4400		4500
			440†			3900†
(b) 8 mos. pregnant	35000			53000		
8. E. G. (a) 5½ mos. pregnant	810		810	3500		4900
			580†			3600†
(b) 7 mos. pregnant	3200			55000		
9. Mrs. S. 3 mos. pregnant	180		520	780		900
			390†			750†
10. Mrs. D. G. 4 mos. pregnant	1060			5300		
11. R. M. (a) 6 mos. pregnant	1500			37000		
(b) 7 mos. pregnant	11000			55000		

* Small amounts of these urines were acidified, boiled and diluted for assay so that the final concentration of HCl was not more than 1.5 per cent. No appreciable loss in potency, however, followed adjustment of the pH to 7–4, *provided the specimens were assayed immediately.*

† For comparison these extracts were taken up in normal saline solution instead of olive oil.

TABLE 1—*Concluded*

SOURCE OF SPECIMEN	OESTRIN RECOVERY—RAT UNITS IN 24° VOLUME					
	Untreated (pH of 7.0 to 4.0) "Free"			Boiled with 15 per cent HCl—"Total"		
	Unextracted	Extracted with ethyl acetate	Extracted with benzene	Unextracted*	Extracted with ethyl acetate	Extracted with benzene
12. Mrs. S. D. (a) 5 mos. pregnant..	1100			5500		
(b) 6 mos. pregnant............	3000			24000		
(c) 6½ mos. pregnant...........	3100			37000		
(d) 8 mos. pregnant............	16000			38000		
13. Mrs. G. 3½ mos. pregnant.......	1000			3500		
14. L. S. 4½ mos. pregnant..........	780			4600		
15. Mrs. M. W. 7 mos. pregnant....	3400			20400		

concentrated hydrochloric acid, 5 to 10 minutes with 15 per cent being sufficient.[2]

If the augmented oestrogenic potency of pregnancy urines after boiling with acid were the result of a transformation of the hormones present into more active structural forms (7), one would expect a constant percentage increase to follow this treatment. The results in table 1 show that this is not the case. In order to be certain of this point, however, the effect of acid treatment was tried upon a pure compound. For this purpose theelin (Parke, Davis & Co.), the crystalline preparation of ketohydroxyoestrin, was used. Three different lots, all over a year old, were tested. When first diluted for injection they were found to contain 40, 30 and 30 r.u. per cc. respectively. When boiled for 10 minutes with 15 per cent hydrochloric acid, they titered at 75, 60 and 50 r.u. per cc. respectively. However, when the untreated, diluted solutions were allowed to stand for 10 days or more before testing, they also contained 75, 60 and 50 r.u. per cc. respectively. Needless to say, this unexpected finding was checked enough times to convince us of its verity. The only apparent explanation is that by long standing in the slightly alkaline medium in which it is

[2] Cohen and Marrian (11), in an article which appeared when this work was practically completed, report briefly their experiments on acid treatment of the urine of pregnant women. They acidified with HCl to a pH of 1 to 2 and found that in order to get the greatest oestrogenic potency without any destruction, it was necessary to autoclave at 15 pounds' pressure for 2 to 4 hours. It seems probable that our method, using a great deal higher concentration of acid and heating for a much shorter time, has the same final effect.

TABLE 2

Effect of varying the amount of acid or the time of boiling upon the yield of "total" oestrin from a pregnancy urine

Each sample represents 6 cc. of the specimen plus acid, heated, and then diluted to 200 cc. for assay.

SAMPLE NO.	HCl ADDED BEFORE HEATING	TIME OF ACTUAL BOILING	TIME IN STEAM BATH AT 100°C.	ACID CONCENTRATION AT TIME OF ASSAY	YIELD OF OESTRIN r.u./24°
	per cent				
1	15	5 minutes		pH 7-4*	78000
2	15	5 minutes		0.45 per cent	78000
3	7.5	5 minutes		pH 7-4*	16000
4	30	5 minutes		pH 7-4*	78000
5	15	1 minute		pH 7-4*	11000
6	15		10 minutes	pH 7-4*	39000
7	15		20 minutes	pH 7-4*	52000
8	15		1 hour	pH 7-4*	78000
9	15	1 hour with reflux condenser		0.45 per cent	78000
10	15		2 hours	pH 7-4*	62000
11	15		10 hours	0.45 per cent	39000

* Neutralized just before assay.

TABLE 3

Values for "free" and "total" oestrin in the urines of a woman during the third month of pregnancy; demonstrating the consistent yields of "total" oestrin, and the quantitative inadequacy of assays on untreated specimens

	URINARY OESTRIN—RAT UNITS IN 24 HOURS		
	"Free" Fresh concentrated urines of pH 7 to 4		"Total" Boiled 5 minutes with 15 per cent HCl benzene extract
	Unextracted	Benzene extract	
2 months pregnant	Less than 45	14	73
7th day of 3rd month	130	37	270
9th day of 3rd month	Less than 66	28	250
11th day of 3rd month	Less than 50	11	320
13th day of 3rd month	58	35	350
15th day of 3rd month	Less than 72	300	340
17th day of 3rd month	150	91	320
19th day of 3rd month	115	130	370
21st day of 3rd month	Less than 70	220	515
23rd day of 3rd month	174	325	650
25th day of 3rd month	280	540	630
28th day of 3rd month	178	380	950
3 months pregnant	185	520	1000
4th day of 4th month	180	190	1340

made up theelin was bound, and that merely by standing after dilution its potency was regained. Acid treatment, therefore, merely immediately broke up whatever combination it was that rendered the original hydroxy-ketone partially inactive.[3] It seems probable that some analogous process occurs in urines. We were unable to find any relation between the pH of urine specimens and the "free" oestrin, but variations in other urinary constituents (depending possibly upon the diet of the individual) might well determine the amount of oestrin demonstrable in untreated urines. We assume for the present, then, that boiling urine with acid does not produce a structural change in the hormone with resultant greater activity,

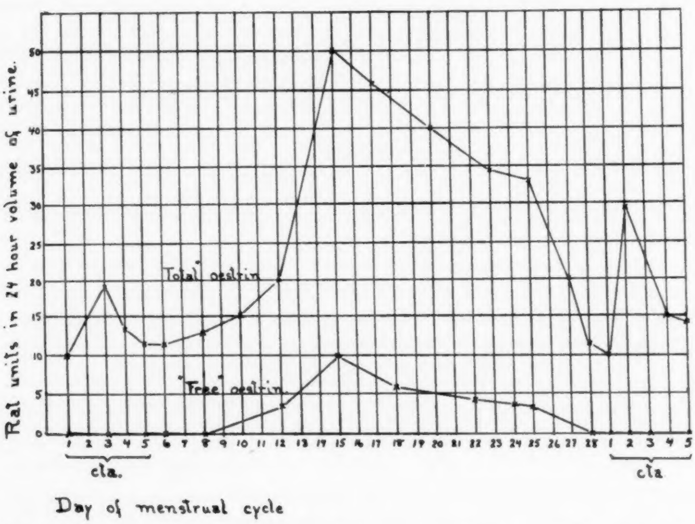

Fig. 2. Demonstrating the difference between "free" and "total" oestrin (ethyl acetate extraction) in the urines of a normal woman throughout one menstrual cycle.

but simply "frees" (possibly through hydrolysis) oestrin which was already present but bound by urinary constituents.

Table 3 strikingly illustrates the importance of acid treatment in the quantitative analysis for urinary oestrin. The urines were all extracted and tested while fresh. They were concentrated specimens and no adjustment of pH was necessary. The values for "total" oestrin follow a uniform curve, rising steadily as pregnancy advanced. The values for "free" oestrin, however, are inconsistent from day to day.

[3] These results have been confirmed by similar experiments with theelol, the crystalline preparation of trihydroxyoestrin (Parke, Davis & Co.), which is also made up in a slightly alkaline solution.

The curves in figure 2 illustrate the difference between "free" and "total" oestrin in the urines of a woman throughout a normal menstrual cycle. Although the values on both treated and untreated specimens show a peak at the 15th day, the curves are only roughly parallel. The ratios vary between 1 to 5 and 0 to 30. We as yet do not know whether or not this is a typical curve. It is presented merely to illustrate the inadequacy of tests on untreated urines and cannot be compared with curves which have been published by others. These analyses were performed before we had adopted the benzene extraction, so that with this better method higher values for "total" oestrin and a greater degree of accuracy may be expected.

In table 4 are the analyses of 14 urines from 12 non-pregnant individuals,

TABLE 4

Comparison of "free" with "total" oestrin in the urines of non-pregnant individuals

NAME	MODE OF EXTRACTION	URINARY OESTRIN—r.u. IN 24° VOLUME	
		"Free" pH of 7 to 4	"Total" Boiled 5 minutes with 15 per cent HCl
1. Mrs. S.	Ethyl acetate	6	60
2. Rosalie S.	Ethyl acetate	6	10
3. Miss V. P.	Ethyl acetate	Negative for 2.5 r.u.	10
4. Mrs. G. H.	Ethyl acetate	2.5	95+
5. Mrs. G. G.	Ethyl acetate	Negative for 2.5 r.u.	Negative for 10 r.u.
6. Mrs. M.	Benzene	20	30
7. Mrs. A. M.	Benzene	20	20
Same patient one week later	Benzene	Negative for 2.5 r.u.	25
8. Mr. R. W.	Benzene	Negative for 2.5 r.u.	15
9. Mr. R. W.	Benzene	75	400
10. Mrs. S.	Benzene	Negative for 2.5 r.u.	10
11. Miss H.	Benzene	60	60
12. Mrs. A. G.	Benzene	20	100
13. Rita S.	Benzene	Negative for 2.5 r.u.	10

both before and after acid treatment. Here again the inconsistency of the ratios of "free" to "total" oestrin are demonstrated. Case histories were purposely omitted, since no clinical significance may be attached even to the results for "total" oestrin until the limits for normal excretion have been established.

The experiments outlined indicate that the amount of oestrin demonstrable in untreated urines depends upon variable interfering substances and not upon the physiological condition of the patient. When urines are tested for "total" oestrin only, the whole specimen may be boiled with acid and extracted. In only one instance (no. 5, table 4), have we found less than 10 rat units of "total" oestrin in the urines of either normal or

abnormal non-pregnant individuals, so that at least 10 animals may be used for assay. Since most specimens contain considerably more oestrin than this, it is hardly ever necessary to extract the whole 24-hour amount.

The final procedure that we have adopted in the analysis of urines from non-pregnant individuals is as follows: A 24-hour volume is collected and the volume measured. We are in the habit of making creatinine determinations upon all specimens. Together with the weight of the patient, this gives a fairly accurate gauge as to whether or not a true 24-hour volume has been collected. For each liter of a 24-hour volume of non-pregnancy urine, 200 to 800 cc., depending upon the amount of oestrin probably present, are measured into an Erlenmeyer flask. Fifteen volumes per cent of concentrated hydrochloric acid are added and the mixture heated to boiling and boiled vigorously for 10 minutes. The material is then transferred to the large extraction flask (see fig. 1). This flask and the smaller one are both filled to the neck with benzene, the apparatus connected and the extraction carried on for 24 hours. At the end of this time the large flask, containing urine and benzene, is disconnected, emptied, put back in place and most of the benzene in the smaller flask distilled over into it. The benzene so recovered contains no oestrin and may be used repeatedly. In the case of large specimens containing small amounts of oestrin, two runs must be made, since the capacity of the apparatus is 1000 cc. The benzene extracts are then combined and transferred to a small beaker, washing with benzene. Six cubic centimeters of olive oil are added and the benzene evaporated off.

In assaying we usually test first with 0.75 cc. of olive oil extract upon each of 2 animals; i.e., for 10 to 40 r.u. of "total" oestrin per 24 hours, depending upon the amount of urine extracted. If negative, progressively larger, and if positive, progressively smaller, amounts of extract are tested upon 2 or more rats at a time until the minimum dose that will produce oestrus in half the animals is discovered. The more oestrin present, therefore, the more rats used, and the more accurate the test. Often the extracts must be diluted with olive oil for the final assays.

Calculation:

$$\frac{(24^\circ \text{ volume} \div \text{cc. extracted}) \times 6}{\text{R}} = \text{r.u. in } 24^\circ \text{ volume}$$

where R stands for the smallest amount of extract that will produce oestrus.

SUMMARY AND CONCLUSIONS

A series of comparative analyses demonstrated that neither chloroform, olive oil, ethyl acetate nor benzene could be counted upon to give complete recovery or quantitatively comparable percentages of oestrin as it occurs

in untreated pregnancy urines. Chloroform gave the lowest and benzene the highest values.

Recovery experiments, employing 24-hour continuous extraction with benzene, revealed that assays on untreated pregnancy urines could not be used as a gauge of the amount of oestrin actually present, since in some cases more was recovered than had been demonstrable in the unextracted specimens.

Pregnancy urines, after being boiled for 5 to 10 minutes with 15 volumes per cent of concentrated hydrochloric acid, increased greatly in their oestrin content. There was, however, no constant ratio between the amount of oestrin found in the untreated specimens and the amount "freed" by acid treatment. The ratios varied between 1:1.5 and 1:40.

Benzene extraction was shown to give as quantitative a recovery of the oestrin "freed" by acid treatment as could be expected when using a biological means of assay. With 3 urines ethyl acetate extracted 12 to 34 per cent less "total" oestrin than did benzene.

Experiments in which the concentration of acid and the time of boiling were varied demonstrated that no greater increase in potency could be produced by changing the technique within certain limits, and that no destruction of "freed" hormone resulted from boiling for 10 minutes with 15 per cent acid. This procedure, therefore, was adopted and the oestrin so recovered called "total."

In assays upon the pure hydroxyketone, theelin, it appeared that upon long standing in the slightly alkaline solution in which it is put up, theelin was bound, since when first diluted for injection the potency was considerably less than could be demonstrated after the diluted solution had stood for 10 days or more. Acid treatment did not result in any further increase in its oestrogenic activity. It was concluded, therefore, that acid treatment did not produce a more potent oestrogenic form but simply "freed" the theelin which was originally present. No relation between the pH of urine specimens and the amount of "free" oestrin could be established, but it seems probable that some analogous process occurs and that the amount of oestrin "free" at any one time in any one specimen depends upon variations in the other urinary constituents.

From repeated analyses over a period of one month upon the fresh, slightly acid urines of a pregnant woman, it was shown that, whereas the values for "free" oestrin were strikingly inconsistent from day to day, the "total" oestrin followed a uniform curve, rising steadily as the pregnancy advanced.

"Free" and "total" oestrin were extracted from the urines of a woman throughout a normal cycle. The curves ran roughly parallel, but the actual ratios of "free" to "total" varied between 1:5 and 0:30.

The results on single specimens from 12 non-pregnant individuals showed ratios of "free" to "total" oestrin varying from 1:1 to 1:35+.

An outline of the exact procedure finally adopted for the assay of oestrin in urines from non-pregnant individuals is given.

It is concluded that this procedure gives physiologically significant values, whereas the usual methods of analyses upon untreated urines may result in completely misleading figures bearing no relation to the amount actually present.

It at first seemed possible that the increase in oestrogenic potency of urines after boiling with acid might be due to the transformation of some physiologically inactive oestrin precursor (e. g., possibly pregnandiol) into an active form. The results tabulated, however, especially in tables 1 and 3, are inconsistent with this interpretation, and indicate that the physiologically significant values are represented by the figures for "total" oestrin only.

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THE EFFECT OF CORONARY OCCLUSION ON MYOCARDIAL CONTRACTION[1]

ROBERT TENNANT[2] AND CARL J. WIGGERS

From the Department of Physiology, Western Reserve University Medical School, Cleveland, O.

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Many students of the coronary circulation must have noted that the ventricular zone affected by ligating a large coronary branch not only appears cyanotic and dilated, but that it seems to alter in its mode of contraction. The detailed and sequential changes in contraction are not easily followed by the unaided eye and so far have not been recorded myographically. The reasons for this were the lack of an adequate and suitable myograph and a technic for the application of one to a limited ventricular surface so that records obtained represent, at least reasonably well, changes in muscle length and not predominantly artefacts due to position changes, thrusts and vibrations of the vigorously beating ventricle. This communication concerns itself with descriptions of a technique and of a type of optical myograph suitable for such studies and an analysis of the changes in optical myograms which follow clamping of a large coronary vessel.

Apparatus. After preliminary efforts to obtain satisfactory ventricular myograms with the segment myograph used by one of us (Wiggers, 1916) to study auricular contraction, it became obvious that in order to overcome the distortions produced by twists and thrusts of the beating ventricle an instrument was needed in which the movable lever arm operates in fixed bearings. A suitable myograph which retains the compactness, lightness and efficiency of the earlier form is illustrated in figure 1. The body of the instrument consists of a small receiving tambour, *E*, (2.5 cm. in diameter) from which a tube leads off at right angles for connection with an optical segment capsule. The lever arms which are of aluminum are spaced 1.5 cm. apart. The rigid arm, *A*, is attached solidly to the back of the tambour and the movable one, *B*, is pivoted in jewel bearings, *C*, as indicated in the insert sketch. The total weight of the myograph is only

[1] The expenses of this investigation were defrayed partly from a grant to one of us (C. J. W.) by the Ella Sachs Plotz Foundation, and partly from a grant to the other (R. T.) by the Josiah Macy, Jr. Foundation.

[2] Fellow of the National Research Council.

10 grams, hence its attachment does not modify cardiac contraction. When the lever arms are securely stitched through the eyelets to the ventricular surface their approximation compresses the tambour rubber, *D*. The pressure changes thus created are transmitted to a Frank capsule optically so arranged that the recorded curve is upward in direction. The myograph arms must be firmly attached to the ventricular surface and exactly aligned in the direction of the superficial muscle fasciculi of the region studied. Twisting action still exerted on the myograph by the contractions of other fibers can generally be eliminated if the rubber tube connection on the lead-off tube is turned in one direction or the other until such motions are gone. To minimize the transference of finer vibrations, such as heart sounds, the tambour is covered with a relatively thick rubber dam (0.29 mm.) and the Frank capsule with a dam as heavy as the registration of curves of proper amplitude permits.

Fig. 1. Diagram illustrating ventricular myograph for use with optical capsule. *A*, fixed lever arm; *B*, movable lever arm, mounted in jewel bearings at *C*. *D*, rubber dam covering small tambour, *E*, with right angle lead-off tube. (Actual size.)

The attached myograph is suspended by an elastic band, the tension of which must be meticulously adjusted so that a light pull is exerted upon the stitches and underlying myocardium. With proper adjustment of the tension the up and down movements of the heart as a whole are not recorded. If the size of the ventricle changes, readjustments of tension can be made conveniently by means of a screw control at the upper end of the elastic suspension.

In addition the exposed heart is so adjusted in a pericardial cradle that the region selected for study moves as little as possible. The central anterior surfaces of both the left and right ventricles are obviously most suitable for recording good myograms, although even the extreme apex and basal regions with their extensive movements yield satisfactory records.

Methods. Dogs 10 to 20 kilos in weight were anesthetized with morphine and sodium barbital and under mild artificial respiration the heart was exposed and rested in a pericardial cradle. In most experiments the *ramus descendens anterior* of the left coronary artery was isolated within 2 cm. of its origin in preparation for the application of a miniature clamp, and the myograph was stitched to the central anterior surface of the left ventricle. In a few experiments the right coronary artery

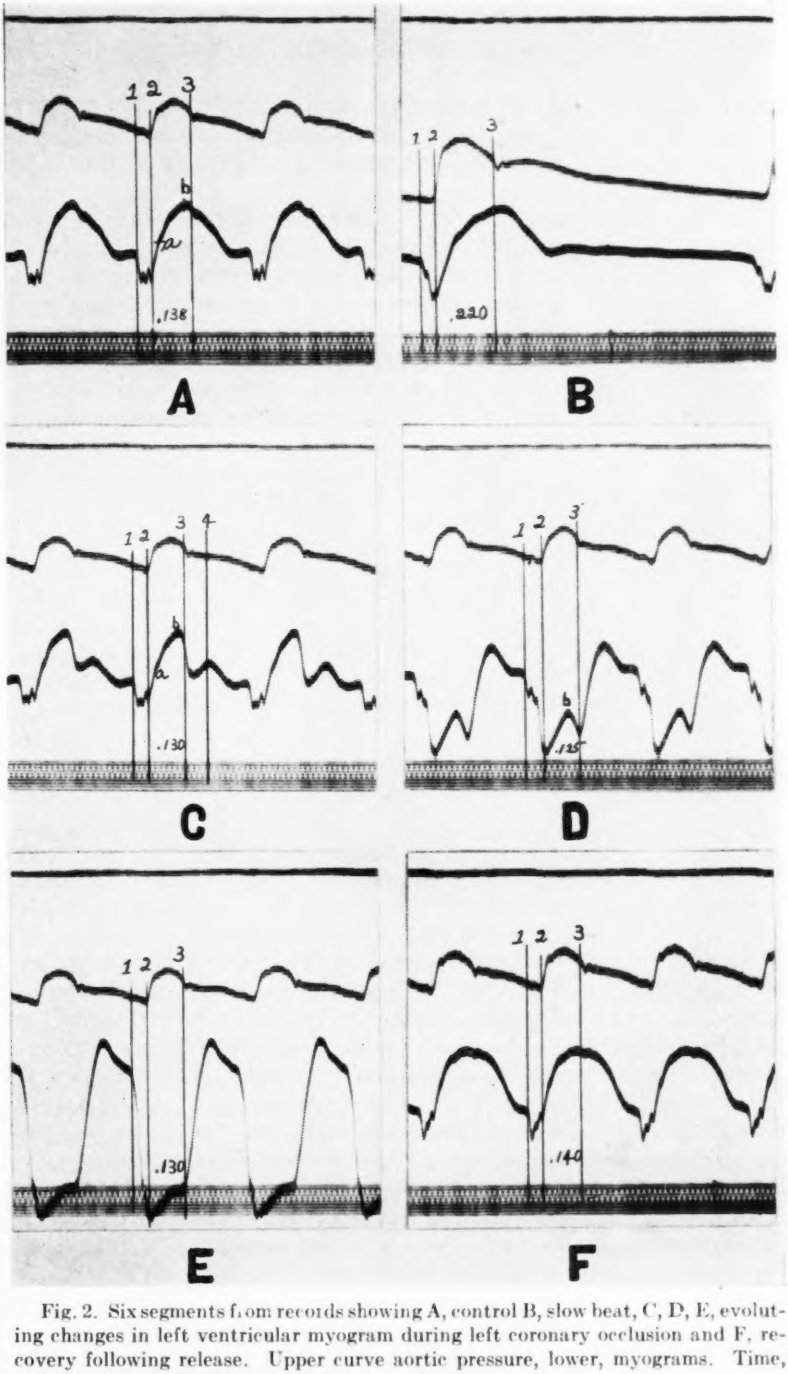

Fig. 2. Six segments from records showing A, control B, slow beat, C, D, E, evoluting changes in left ventricular myogram during left coronary occlusion and F, recovery following release. Upper curve aortic pressure, lower, myograms. Time, 0.02 second. Further discussion in text. (Reduced.)

was isolated instead and the myograph similarly applied to the right anterior ventricular surface. In some experiments a second myograph was attached to a region of ventricle not supplied by the coronary vessel to be occluded.

In connection with such optical myograms, pressure pulses from either the aorta or the left ventricle were recorded in the usual manner by means of calibrated optical manometers. After satisfactory control records had been obtained the isolated coronary vessel was securely clamped and synchronous optical records were taken either continuously on slowly moving paper or at frequent intervals after occlusion on rapidly moving paper. In a similar fashion the effects of decompression of the coronary artery were also studied after varying intervals of occlusion. During

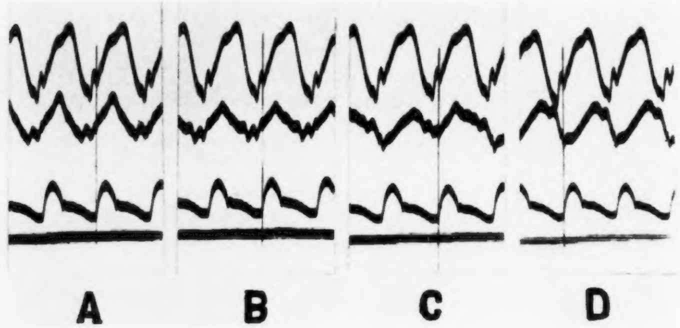

Fig. 3. Four segments of records showing A, control B, C, D, evoluting changes of right ventricular myogram (middle) and unaltered character of left ventricular myogram (upper) following ligation of right coronary artery. Lower curve, aortic pressure. (Reduced.)

several experiments, ventricular fibrillation occurred either before or just after the release of the clamp. In a large proportion of such accidents fibrillation was abrogated and a perfectly normal beat reëstablished by applying electrodes to the heart and sending brief counter-shocks of an A-C current directly through it, as described by Hooker, Kouwenhoven and Langworthy (1933).

Characteristics of the normal ventricular myogram. Myograms recorded from different accessible regions of the right and left ventricle are essentially alike. Typical tracings from the left ventricle are shown in figure 2, A and B, in which curves with rapid and slow rates of beat are illustrated. Other examples are shown in figure 3 (upper curve) and in curve *A* of figure 4.

During the isometric contraction phase (indicated by lines *1–2*) the

myograms show two characteristic deformations, i.e., either a steep drop followed by several small oscillations or a transient sharp positive spike. Frequently less conspicuous oscillations or dips characterize this phase. All of these must be considered as artefacts which we have not succeeded in eliminating.

Precisely with the onset of ejection (line *2*) the myogram at first rises steeply (*2-a*) and then more gradually to a summit near or at the end of systole (line *3*). This portion of the curve corresponds to the auxotonic shortening process. The summit generally persists during isometric relaxation (fig. 2, *B* and *F*) or sometimes a further small elevation follows the incisura which is an artefact (fig. 4, *A*). With the onset of ventricular filling the curve declines rather rapidly to a basic diastolic level. In cycles with a long diastole (fig. 2, *B*) a slight gradual after-stretching occurs during diastasis.

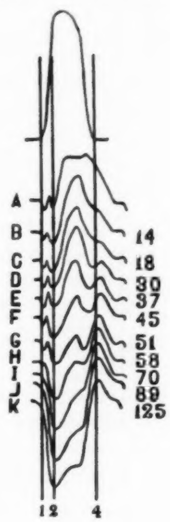

Fig. 4. Series of transcribed left ventricular myograms from a continuous record (*A–K*) showing sequential changes following ligation of left ramus descendens, in relation to a left ventricular pressure curve (upper). Numbers to right denote number of beats following occlusion. Discussion in text.

Changes in myograms following occlusion of ramus descendens anterior. Continuous records taken after occlusion show a series of evoluting changes in the contour of the contraction curve leading in about a minute to its complete inversion during systolic ejection (fig. 2, *E*). The detailed evolution is shown in curves of figure 4. Although the myograms alter from beat to beat they may for convenience be described as of three categories, viz., 1, an initial type characterized by smaller amplitude and decreased duration of contraction (fig. 2, C; fig. 4, *B–F*); 2, transitional types (fig. 2, *D*; fig. 4, *G*, *H*, *I*) indicating progressive decrease in shortening and a struggle between the forces causing shortening and those tending to lengthen the fibers, and 3, frankly inverted myograms (fig. 2, *E*; fig. 4, *J–K*). A detailed study of myograms recorded immediately following occlusion shows in addition to a decreasing amplitude of contraction a concomitant shortening of the period of contraction of the affected muscle (*2-b*) as illustrated in A, C and D of figure 2. The duration of systolic ejection also decreases during this interval and usually remains reduced until some time after frank inversion of the myogram has occurred when it may again come to equal the initial duration.

Analysis of the inverted myogram (fig. 2, *E*; fig. 4, *J–K*) brings out several significant changes in time relations. The chief abrupt drop of the curve,

indicating expansion of the ischemic muscle, occurs during the isometric rise of intraventricular pressure and prior to the rise of aortic pressure. During systolic ejection (*2–3*) a slight degree of muscle shortening generally occurs; the curve is rarely an inverted image of the intraventricular pressure summit. Precisely synchronous with the incisura of the aortic pressure curve (*3*) and the rapid fall of intraventricular pressure during isometric relaxation the curve rises sharply to the diastolic level with a subsequent gradual decline as the ventricle fills during diastole.

TABLE 1

EXP. NO.	DURATION OF LIGATION	TIME INTERVAL BETWEEN LIGATION AND FAILURE	RECOVERY	TIME INTERVAL BETWEEN RELEASE AND RECOVERY	REMARKS
1	1 min. 40 sec.	50 sec.	Yes	45 sec.	Without fibrillation
2	2 min. 20 sec.	50 sec.	Yes	30 sec.	Without fibrillation
3	2 min. 30 sec.	35 sec.	No		Fibrillation without revival
4	2 min. 30 sec.	20 sec.	No		Fibrillation without revival
5	3 min.	75 sec.	No		Fibrillation without revival
6	3 min.	150 sec.	No		Fibrillation without revival
7	4 min.	55 sec.	Yes	11 min.	Fibrillation revived
8	4 min. 44 sec.	90 sec.	Yes	1 min. 40 sec.	Without fibrillation
9	4 min. 45 sec.	70 sec.	Yes	14 min.	Fibrillation revived
10	10 min. 30 sec.	40 sec.	Yes	5 min. 30 sec.	Fibrillation revived
11	23 min.	65 sec.	Partial	8 min.	Fibrillation revived
12	45 min.	150 sec.	No		Heart beat for 2 hours after release
13	46 min.	80 sec.	No		Fibrillation without revival
14	1 hr. 50 min.	31 sec.	No		Fibrillation 14 minutes after release and revived for 8 minutes

These changes persist until the ventricle fibrillates or stops in a hypodynamic state as is usual in most acute experimental occlusions. In some instances, however, as in the series reported by Orias (1932) an effective ventricular action continued for 1 to $1\frac{1}{2}$ hour despite the constant lack of any significant contraction in the ischemic zone.

When in such experiments the coronary clamp is released after a short period of closure, a reversed set of evoluting changes occurs leading rapidly to a complete restoration of normal contractions in the zone (fig. 2-*F*).

A determination of the maximal duration of ventricular ischemia compatible with such recovery requires a much larger series of observations than are at our disposal at present, although the data of table 1 indicate that after intervals of 23 minutes or greater, recovery was only partial or absent even an hour later.

The excitability and conductivity of the ischemic area. The absence of contraction in the ischemic zone may be due either to a depression of the contractility of the affected muscle or to a failure of impulses to reach it because of a loss of conductivity and/or irritability. That these latter functions are not essentially disturbed was demonstrated by the following experiments:

Myographic records were simultaneously recorded from the right and left ventricles. The anterior descending ramus of the left coronary was ligated and after several minutes when the myogram of the left ventricle showed distinct inversion the heart was stopped by vagal stimulation and the ischemic zone stimulated with rhythmic break induction shocks of a strength which had previously been found just adequate to excite the normal ventricle. With this stimulation impulses spread in the usual manner to the right and left ventricles and caused their contraction, although each elevation of left intraventricular pressure continued to expand the ischemic area. This demonstrates that the functions of contractility can be seriously impaired by anoxia while the properties of irritability and conductivity are essentially unchanged.

The ineffectiveness of perfusions with Locke's or Tyrode's solutions. In an effort to evaluate the minimal oxygen supply capable of sustaining muscular contraction it was proposed to perfuse the descending ramus of the left coronary artery with fully oxygenated Locke's or Tyrode's solutions and then by progressive gradual decreases in oxygen pressures and simultaneous time flow measurements to calculate the minimal oxygen requirement necessary to sustain contraction. Since no difficulty was encountered by us in maintaining beats in the hearts of small dogs perfused by the Langendorff method with the same solutions and since various workers in this laboratory had shown that fibrillation and hypodynamic failure so common in dogs after coronary ligation could be prevented by such perfusion even at low pressures the plan seemed perfectly feasible. It immediately became apparent, however, that even at the highest oxygen and perfusion pressure compatible with experimental methods (200 mm. Hg above atmospheric pressure) such solutions are certainly incapable of maintaining sufficient contraction in the perfused zone to cause a shortening with the heart performing work under natural conditions. The myographic curves from these zones invariably resembled those of figure 2, E. Since it can be shown by simple calculations that such solutions even under these high O_2 tensions cannot absorb more than 2.5 volumes per cent of

oxygen, coronary flow cannot be made great enough to supply sufficient oxygen for supporting efficient contractions in the normally working heart. Further studies employing pure hemoglobin solutions will be required to determine the minimal oxygen requirements.

The responses of the right ventricle during ischemia. Whether the contractile failure will eventually prove to result from exhaustion of and a failure to resynthesize phosphocreatine, from accumulation of lactic acid, from decrease in pH, or less probably from failure in oxidation of lactic acid there can be no doubt but that anoxia due to an inadequacy of collateral blood supply is the tangible factor. Inasmuch as the Thebesian and other communications are anatomically more extensive in the right heart and since its total metabolic requirements are less, the question arises whether a similar prompt reduction and failure of efficient contraction occurs after occlusion of the right coronary artery.

To study this question the right coronary artery was isolated and compressed and the effects on myograms recorded from both right and left ventricles were compared. Such experiments illustrated by 4 segments of records in figure 3 demonstrated that changes identical with those described in the case of the left ventricle are certain to follow occlusion of the right coronary artery, although the time required for complete reversal was regularly somewhat longer (up to 3 or 5 minutes). In these records the upper curves are myograms from the left ventricle, the middle curves, myograms from the right ventricle, and the lower, an aortic pressure curve. The right myograms in segment B, C and D show respectively the initial depressed, the transitional and the frankly inverted characteristics, while the character of the left ventricular myogram remains unaltered. Apparently, the advantages gained by the better collateral communications of the right coronary system with the ventricle are largely offset by the fact that they necessarily carry less completely oxygenated blood than do the main vessels.

Discussion. The interesting and somewhat surprising discovery that approximately one minute after coronary occlusion the contractile force in an ischemic area is either abrogated or certainly so feeble that the ischemic muscle stretches instead of shortens during systole and in proportion to the elevation of ventricular pressure, has many implications of importance to clinical medicine and experimental physiology. Of these we shall discuss a few:

1. Our results demonstrate more convincingly than direct circulatory studies the functional inadequacy of anatomically described collateral branches to ventricular muscle. Furthermore, the numerous instances of infarction and cicatrization found postmortem after coronary occlusion in humans and in dogs (experimentally produced) indicate that circulatory conditions are not dissimilar in these hearts. Consequently our observa-

tions strongly suggest that if an extensive collateral circulation has not developed prior to a total occlusion, the muscle in the zone affected is not likely to survive.[3]

2. Since the muscle fibers in the affected zone promptly undergo periodic stretching instead of shortening, the thought arises that such mechanical factors, rather than chemical, as commonly postulated, may be the ultimate stimulus to the sensory nerves and so account for the immediate intense pain associated with the occlusion. Upon such an assumption, the hitherto unexplained benefits of pressure lowering drugs would be clarified since, by lowering the maximum intraventricular pressure, the degree of the periodic stretching would be reduced. This possibility is worthy of more extensive investigation.

3. Experimentally our observations supply tangible proof for certain logical assumptions that Orias (1932) while working in this laboratory was obliged to make in order to explain the pressure changes immediately following coronary occlusion. To account for the immediate reduction in duration of ventricular systole, this investigator postulated a prompt reduction of contractile power in the ischemic area. In order to account further for the negligible decline of systolic pressure or in some instances its complete absence before compensatory reactions of other regions had had time to develop required the further assumption that the contractions in the ischemic area were of shorter duration. Our observations have demonstrated the occurrence of both conditions. Myographic curves have shown that localized anoxemia produces the same abbreviation of contraction in the affected zone that generalized anoxemia does upon the whole heart (Sands and DeGraff, 1925). No evidence was obtained however that it exerts an initial increase in contraction as seemed to follow from studies of general anoxemia.

Our observations that a marked systolic expansion of the ischemic region replaces shortening makes it evident that a considerable fraction of the total pressure developed is lost in producing such distention. We would therefore supplement Orias' theoretical analysis of fundamental mechanisms by adding the suggestion that the hypodynamic levels which so often occur despite a rise of initial tension may not necessarily be due to a fatigue of the remaining contraction fractions, but can be accounted for by the loss of pressure in expanding the regions in which contractions are enfeebled or absent.

4. The myographic study of localized ventricular areas which we have

[3] Further experimental support for this conception has been obtained since this paper went to press. Inasmuch as a free collateral flow might conceivably be prevented by occlusion, the peripheral end was incised so that a free flow of any blood from collateral sources could occur. This was found to be without effect either in preventing or delaying the loss of contractile power.

introduced and a knowledge of the sequential changes that follow deprivation of blood supply should prove useful in estimating the value of drugs in coronary occlusion, both those that might insure a better collateral supply and those that might act through a direct effect on the muscle. Unless it can be shown that some degree of immediate improvement in contraction occurs in the regions affected, no great practical value as regards maintenance of function and avoidance of subsequent pathological changes can be anticipated.

SUMMARY

1. An optical myograph suitable for recording localized contractions from a ventricular surface and a technique for its correct application are described.

2. Normal myograms recorded simultaneously with aortic or ventricular pressure curves, though slightly deformed by oscillations during the isometric contraction and relaxation phases clearly show the natural shortening which occurs during ventricular ejection and the lengthening which follows isometric relaxation.

3. Occlusion of a main coronary branch is followed by an evoluting series of myographic changes which indicate progressive enfeeblement of contraction to the extent that approximately within a minute the area stretches during isometric contraction, remains stretched during systolic ejection and shortens quickly during isometric relaxation; in short, the myogram is completely inverted. Similar changes in contraction of the right ventricle occur following ligation of the right coronary artery. These observations demonstrate convincingly the functional inadequacy of described collateral circulation in normal hearts.

4. Reëstablishment of the normal blood supply is followed by a reversed series of myographic changes with restoration of normal vigorous contractions provided the period of ischemia is not too long in duration.

5. Failure of shortening is due to enfeeblement or abrogation of contraction and not to failure of impulses to reach the areas involved, or to excite them.

6. The oxygen requirements for maintaining efficient contractions in the normally working heart are high as evidenced by our failure to maintain efficient contractions when an area is perfusing with highly oxygenated Locke's solution.

7. The observations supply tangible proof for the correctness of Orias' hypothesis that coronary occlusion produces an early abbreviation of total ventricular systole with little or no decline of systolic pressure through a progressive decrease in amplitude and duration of contraction in the ischemic area. Our results suggest further that the tendency for development of hypodynamic ventricular beats following coronary occlusion may

not necessarily be due to fatigue of the remaining contracting fibers, but can be explained by loss of pressure in expanding the regions in which contractions are enfeebled or absent.

8. Several clinical implications of our results are briefly discussed.

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PHASIC VARIATIONS IN PERIPHERAL CORONARY RESISTANCE AND THEIR DETERMINANTS[1]

DONALD E. GREGG, HAROLD D. GREEN AND CARL J. WIGGERS

From the Department of Physiology, Western Reserve University Medical School, Cleveland, O.

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Information regarding the successive changes of resistance in the coronary branches and their determinants is of paramount importance both in understanding the phasic changes of coronary flow and in interpreting the actions of drugs upon the intact coronary circulation. It seemed that a study of the pressure pulse from the peripheral end of a coronary vessel (peripheral coronary pressure—P.C.P.) should supply this information.

This report deals with a description of such pulses recorded by a calibrated optical manometer together with an analysis of their phasic variations and of their reliability in appraising the variations of peripheral coronary resistance under natural conditions.

Procedure. In a first series of experiments aortic and peripheral coronary pressures were simultaneously recorded by two optical manometers (Wiggers pattern). The cannula of the coronary manometer—modified as illustrated in figure 1A—was inserted into the peripheral end of the ligated descending ramus as indicated in figure 1A, the precautions emphasized by Wiggers and Cotton (1933) being observed. Through the lateral connection and stopcock (*a*) the peripheral coronary vessel was perfused with Locke's solution, except when records were being taken. Experience had taught us that by this expedient coagulation in the cannula tip could be prevented and the danger of the heart failing through fibrillation or hypodynamic action could generally be averted.

During the course of such studies the discovery was made by Tennant and Wiggers (1935) that an area so perfused extends during systole instead of shortening. Since replacement of a normal systolic shortening by an extension might conceivably alter the peripheral coronary resistance changes, a second method for recording P.C.P. was devised by which normal contractions were retained in the area studied. The procedure consisted in isolating the anterior descendens or the circumflex ramus and also a suitable side branch and in tying the coronary cannula into the

[1] The expenses of this investigation were defrayed from a grant by the Ella Sachs Plotz Foundation.

latter, as illustrated in figure 1B. The normal blood supply to the area studied was thus kept intact, except while taking records, when the main vessel was clamped centrally to the side branch. Coagulation was prevented in the cannula by flushing it repeatedly with Locke's solution to which heparin had been added. Myographic records showed that such admixture of heparinized Locke's solution had no effect on contractions in the areas studied. By compressing the main branch for several minutes, records could also be obtained when the area supplied did not shorten. By releasing the clamp and restoring the blood supply, normal contractions returned in the area affected as described by Tennant and Wiggers (1935).

In order to improve the "figure of merit" of the manometer equipped with a relatively small cannula tip, a very tense rubber membrane was used. The resulting decrease in sensitivity was compensated for by moving the photokymograph away to a distance of 2.6 meters. Adequate

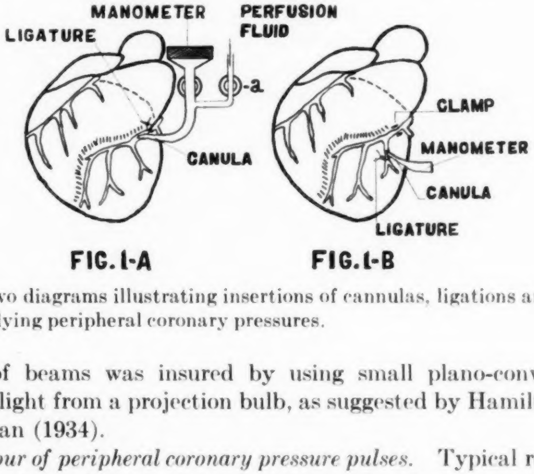

FIG. 1-A FIG. 1-B

Fig. 1. Two diagrams illustrating insertions of cannulas, ligations and clampings used for studying peripheral coronary pressures.

intensity of beams was insured by using small plano-convex mirrors flooded by light from a projection bulb, as suggested by Hamilton, Brewer and Brotman (1934).

The contour of peripheral coronary pressure pulses. Typical records from a heart in which the zone determining peripheral pressure changes was presumably extending during systole (method 1) are reproduced in figure 2. The form of the aortic pressure curves and the ordinate values derived from application of a calibration-scale attest to the existence of good dynamic conditions. Record A was obtained shortly after cannulation of the peripheral coronary branch. Vertical intercepts facilitate comparison of the phasic relations of the two pressure pulses. The curves show that 0.046 second before the steep rise of aortic pressure, i.e., probably coincident with the isometric rise of intraventricular pressure, the peripheral coronary curve rises, slowly at first (*A–B*), then brusquely (*B–C*). This rise continues more gradually into the period occupied by the steep

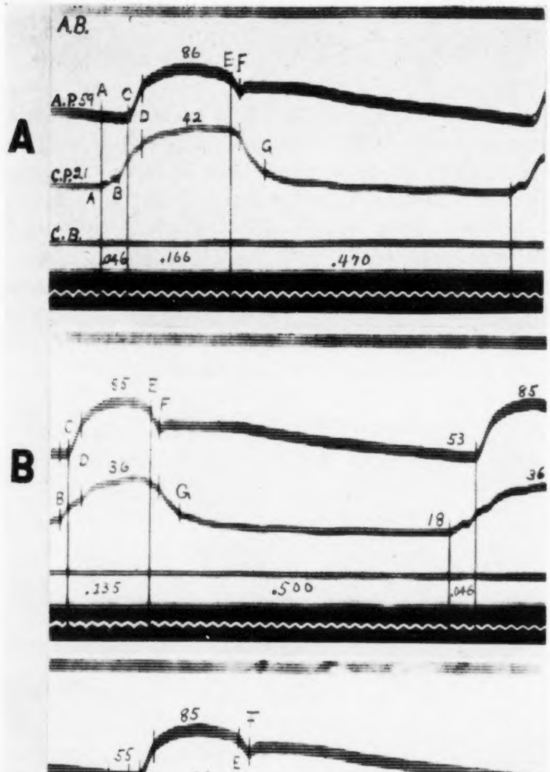

Fig. 2. Records illustrating time relations, contour and magnitude of peripheral coronary pressures. A, normal control; B, during clamping left circumflex ramus; C, after release of same; and D, during clamping of right coronary artery. *A.B.*, aortic base line; *A.P.*, aortic pressure pulse; *C.P.*, peripheral coronary pressure; *C.B.*, coronary base line. Time, 0.02 second. Discussion in text (expt. DD-1/5-8).

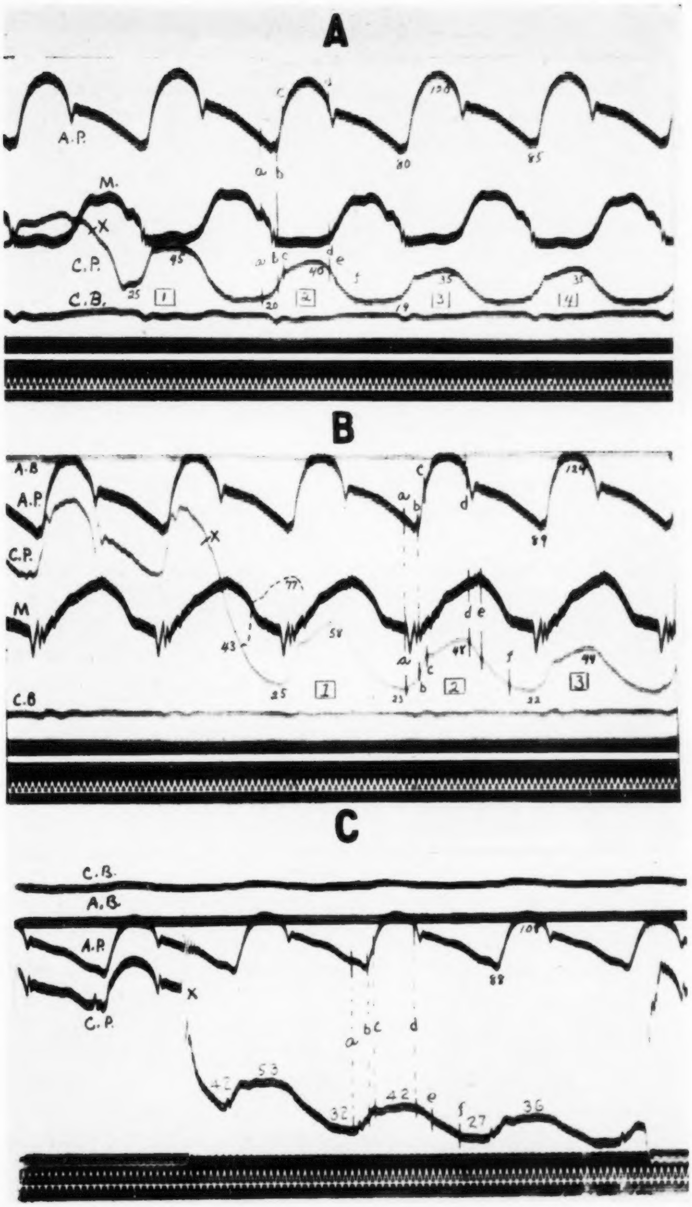

Fig. 3. Comparison of P.C.P. curves from anterior descendens ramus (A) when area extended during systole, and (B) when same area shortened. (C) record from left circumflex when area contracted. *M*, myogram; *X*, time of clamping main coronary; other lettering as in figure 2 (expt. DD-32/6-7; DD-36/10).

rise of aortic pressure (*C–D*), then mounts as a gradually rising plateau almost but not quite to the end of systole (*E*). During protodiastole (*E–F*), the curve starts to decline. At first it falls rapidly, then more slowly during the isometric relaxation phase (*F–G*). At the time ventricular inflow starts (*G*), the fall in pressure is practically completed.

The time relations and general features of the rise and fall correspond to changes of pressure within the left ventricle, but the gradually rising plateau differs essentially from the rounded summit of intraventricular pressure which may be judged from the aortic pressure summit. With minor differences these general characteristics were found in all records. In some instances the abrupt initial rise (*B–C*) extended through the period of rising aortic pressure (*C–D*). The gradient of the systolic plateau also varied in different experiments.

Examples of records in which the area was stretched (A) and in which it was shortening during systole (B), (method 2), are shown in figure 3. Record A was taken after the main branch of the *ramus descendens* had been clamped for 3 minutes, i.e., after sufficient time had elapsed to cause marked extension of the area supplied.[2] This extension is demonstrated by the myographic record which descends abruptly during isometric contraction (*a–b*) and rises with isometric relaxation (*d–f*). The peripheral coronary pressure pulses (beats *2*, *3*) show essentially the same features as those described above. Immediately after taking this record the coronary clamp was removed and the area allowed to recover. Record B in which the myogram now shows a continuous shortening during ejection (*c–d–e*) and slightly beyond it, was then recorded.

The coronary curve of this latter record starts with two beats representing lateral coronary pressure. They serve the purpose of assuring adequacy of the manometer and proper alignment between the manometer and artery. At *X* the main ramus was clamped abruptly and from this point on peripheral pressure changes were recorded.

The corresponding beats marked *2* or *3* in records A and B are very similar, the only difference noted upon close examination of record B being 1, a somewhat steeper rise of the plateau continuing to the very end of systole; 2, a somewhat slower gradient of decline during isometric relaxation; 3, a higher pressure maximum during systole, and 4, a trifling increase in diastolic pressure. If we accept such differences as significant it might be inferred that the existence of local contractile forces tends somewhat to augment the pressure rise during the ejection phase and to retard the decline of pressure during early diastole. In some experiments however even such differences were not apparent, while in others the initial

[2] Just before taking the record the vessel was distended by perfusion with Locke's solution through the side cannula and the point *X* on the record denotes the moment when it was stopped.

sharp elevation of pressure appeared to shift from the isometric period to the time that the aortic pressure rose. It should be noted however that in some experiments of our first series a steep rising plateau was present. It is significant that in all experiments the rise of coronary pressure (*a–b*) definitely preceded shortening as inscribed by the myogram at *c* and that the decline of peripheral coronary pressure definitely preceded the actual lengthening of the muscle (*e*) and began coincidently with the incisura (*d*), i.e., with the fall of intraventricular pressure.

Record C of figure 3 is added without further description as evidence that curves similar in contour can be recorded from the peripheral end of the *circumflex ramus* (method 2). Although a myogram could not be conveniently recorded there is every reason to believe that the region affected continued to shorten, for no compression of the vessel had been made previous to this test.

The determinants of phasic variations in peripheral coronary pressure. The systolic increase in peripheral coronary pressure may be due *a*, to increased intramural or intraventricular tension; *b*, to compression of intramural vessels by shortening and thickening of muscle elements, or *c*, to transmission of pressure from collateral vessels.

In favor of the concept that *tension change* is the predominant factor are the discoveries 1, that the sharp rise and fall of peripheral coronary pressure coincide respectively with the steepest rise and fall of intraventricular pressure, but are not synchronous with the onset of shortening or lengthening recorded myographically, and 2, that as long as intraventricular pressures do not alter, the contour of the curves does not differ essentially regardless of whether the region shortens or lengthens during systole. The fact that the systolic maximum is less when the area supplied lengthens during systole (as in ischemia) can be interpreted to mean either that muscle shortening (and thickening) is normally of supplementary assistance or that stretching increases the capacity of the coronary vessels sufficiently to prevent a full development of systolic pressure (see below).

Before we may conclude that tension changes are chiefly concerned and that length changes play at most a subsidiary rôle, it is necessary to evaluate the part that pressure transmitted through collateral anastomoses with other branches may play. The magnitude of the peripheral collateral circulation in the normal heart is still unsettled, despite extensive anatomical and experimental studies. Experimental studies can easily be cited in favor of either an abundant or a negligible collateral circulation. For instance, if mean pressures are simultaneously recorded from a peripheral coronary branch, and from the aorta, the former is approximately one-fifth that in the aorta; and if aortic pressures are caused to rise by any of several methods this proportionality is roughly maintained. We have

studied such relationships extensively and have even attempted their use in evaluating the effect of drugs on the collateral supply of blood to infarcted areas. We slowly came to realize, however, that it is hazardous to conclude that such correspondence denotes a cause and effect relation, for the dynamics of the left ventricle is modified whenever aortic pressure alters (Wiggers, 1928). That increased ventricular contraction with its concordant increase of the intraventricular pressure maximum is indeed the dominant factor is shown in the record of figure 4, in which aortic and peripheral coronary pressures were recorded while the aorta was being compressed. The rise affects chiefly coronary systolic pressure; diastolic pressure is raised only to an insignificant extent. More important, however, is the fact that the higher systolic pressure occurs chiefly through a greater initial rise which precedes the elevation of aortic pressure, hence

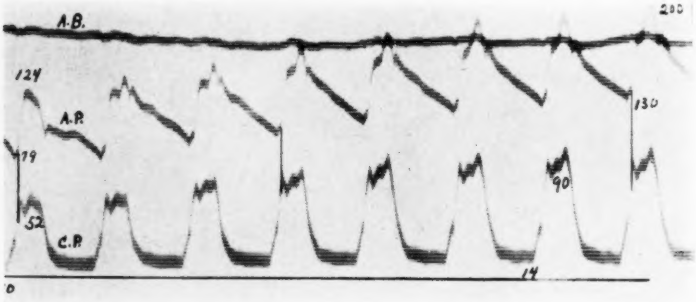

Fig. 4. Aortic (upper) and P.C.P. (lower) showing effect of aortic compression. Discussion in text (expt. DD-5).

the change could not be assigned to transmission of pressure from collateral branches. Opposed to the idea that a significant collateral supply exists are *a*, the recent observation of Tennant and Wiggers (1935) that recognizable contractions in the ischemic area discontinue approximately within a minute after occluding a main branch, and *b*, our own observations that the flow of blood from the peripheral end of a major coronary vessel is extremely small. In the experience of workers in this laboratory, only an occasional drop flows from a cannula in the peripheral end of the *anterior ramus descendens* of the dog, the total flow per minute being less than 1 cc. Flow rates which even approach those reported by Anrep and Häusler (1928) have never been encountered. These must have been recorded from a hound with unusually developed collateral circuits.

Our analysis of optical tracings (e.g., fig. 2A) precludes attributing the rise of peripheral coronary pressure prior to ejection (*A–C*) to trans-

mission of pressure from collaterals. The possibility does exist however that the rising systolic plateau (*D–E*) may be due to such a transfer of collateral pressure. The fact that this slope increases both when the areas contract and when aortic pressures mount (fig. 4) might be cited to support such a possibility.

More direct evidence on these points was obtained by studying changes in P.C.P. in the *anterior ramus descendens* following additional temporary ligation of the left circumflex and/or right coronary branches. Such studies were complicated by immediate changes in contraction of the ventricles and by the rapid development of ventricular fibrillation. Despite such difficulties and numerous expected failures, successful results were obtained in 5 dogs. The records of figure 2 illustrate the results of one such experiment. Record A represents a control which has already been analyzed. Without disturbing the manometers, the left circumflex branch was abruptly occluded by application of a special clamp. Record B was taken as quickly as possible thereafter. The aortic pressure curve displays typical effects of occlusion described by Orias (1932, 1934), among them the relatively small fall in aortic blood pressures and the marked reduction in duration of systolic ejection while the heart cycle remains constant. The coronary diastolic pressure decreased 3 mm. and systolic pressure, 6 mm. After occlusion of less than a minute the clamp on the left *circumflex ramus* was released and within another minute curve C was taken. It is practically identical with that of curve A as regards contour, amplitude and time relations. Then, the right coronary was similarly occluded near its origin and curve D taken. It shows no significant changes of any sort.

These and many similar tests showed clearly that compression of the right coronary artery is without effect upon peripheral pressure in the *ramus descendens anterior*; occlusion of the left circumflex branch on the other hand reduces coronary systolic pressure and modifies the form of the curve. The bulk of evidence distinctly favors the view that the changes noted result chiefly and perhaps entirely from altered contraction of the left ventricle, for 1, the lowered coronary systolic pressure (record B) is chiefly due to a smaller initial rise which transpires before any transmission of collateral pressure could have taken place, and 2, the systolic plateau shows an actual increase in the rate of rise, not a decrease, as would be expected if a collateral transfer of pressure had been abrogated.

The slightly rising plateau therefore remains difficult to interpret. The possibility must still be considered that it represents a slight transfer of pressure directly from the ventricular cavities. Such an interpretation would give a function to the communicating channels described by Wearn et al. (1933) and would account for the appearance in capillaries of particulate matter injected into the ventricles when the coronaries are perfused

from an extraneous source (Bohning, Jochim and Katz, 1933), without necessarily demonstrating the efficiency of such a circulation.

On the basis of such studies the conclusions are reached that the transfer of pressure from collateral vessels plays no significant part in determining the contour or magnitude of peripheral coronary pressure pulses and that the systolic increase in peripheral coronary pressure is chiefly due to muscle tension rather than to changes in muscle length.

Peripheral coronary pressure variations and the appraisal of peripheral coronary resistance. Numerical values for the P.C.P. changes expressed in millimeters mercury and referred to zero at the aortic cannula tip are inscribed directly on curves presented in our illustrations. They indicate the order of magnitude generally found, although considerable difference occurs, particularly in the systolic maximum pressure. Such values doubtless represent the actual maximal pressures developed peripherally to an occlusion under given dynamic conditions. If, however, they also indicate the extreme magnitude of the systolic increase in resistance under normal conditions, then the systolic pressure-difference in the large coronary vessels is great enough to cause a much larger systolic flow than observations by flow-recorders have indicated (Wiggers and Cotton, 1933). A suspicion that such inferences are questionable was aroused by the observations of Anrep and Saalfeld (1933) that when auto-perfused coronary vessels are briefly clamped during systole, the peripheral pressure holds at far higher levels than indicated by our figures. While we have found by repetition of their experiments that the holding level is always distinctly below aortic systolic pressure, it greatly exceeds the pressure maxima indicated in direct P.C.P. curves. Confirmatory evidence is given in the observations that after sudden compression of a coronary branch, the peripheral systolic pressure developed in subsequent beats depends upon the diastolic level from which they start. Thus in the record of figure 3 B, the systolic height decreases progressively in beats 1, 2 and 3. Observations in other experiments showed that even a greater systolic elevation of the curve occurred in still earlier beats, as, for example, the one sketched upon the record. If such beats are enlarged by projection and then redrawn to identical coördinates, their form is found to be unchanged. Such results clearly show that the degree to which the coronary vessels and their branches are filled affects the magnitude of the pressure increment during systole under identical dynamic conditions of cardiac action, but does not alter their form.

The conclusion logically follows that optically recorded curves of P.C.P. picture the *sequential changes in peripheral coronary resistance* correctly as regards time relations and relative magnitude, but they cannot be used quantitatively to appraise the maximum systolic resistance under natural conditions.

We are able to interpret this entirely unforeseen situation in only one way, viz., by postulating a disproportion between the systolic back thrust of blood from the minute vessels and the elastic accommodative capacity of the coronary system involved.

Circumstantial experimental evidence supports this view. The volume-elasticity relations at internal coronary pressures from 20 to 180 mm. Hg were studied immediately after our experiments, in 14 hearts. The method first used consisted in blocking the capillary bed supplied by the *ramus descendens anterior* by perfusion with a dilute suspension of lycopodium. The vessel was connected to a horizontal micropipette and

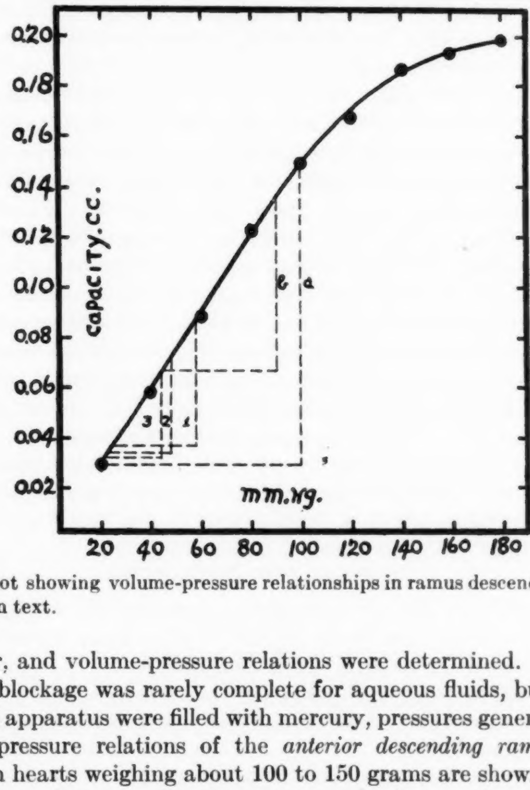

Fig. 5. Plot showing volume-pressure relationships in ramus descendens anterior. Discussion in text.

manometer, and volume-pressure relations were determined. After such treatment blockage was rarely complete for aqueous fluids, but when the vessels and apparatus were filled with mercury, pressures generally held.

Volume-pressure relations of the *anterior descending ramus* and its branches in hearts weighing about 100 to 150 grams are shown by a composite curve in figure 5. Examination reveals that a volume increase of about 0.12 cc. is required to produce a pressure rise from 20 to 100 mm. (line *a*). Similar tests with the optical manometers used showed that introduction of 0.0035 cc. sufficed to cause a similar rise in pressure. Applying such figures it becomes apparent that a pressure of 100 mm.

could only develop in the occluded coronary vessels provided the volume increased at least 0.12 cc. and that registration of the full change by our manometer would require only a trifle more, i.e., a total volume of 0.1235 cc. The only source for this added blood in occluded coronary territory would be by squeezing blood back from minute vessels during systole; a supply from collaterals can at once be eliminated for the major elevation of pressure precedes the rise of aortic pressure. By drawing on the curve of figure 5 vertical lines, *1*, *2*, *3*, the lengths of which correspond to the pressure increases shown in similarly designated beats of figure 3, it becomes apparent that the capacity change and presumably the systolic backflow is of the order of only 0.03 cc.; further that only slight differences in diastolic coronary pressures (i.e., distention) are required to increase the magnitude of the backflow denoted successively by lines *3*, *2* and *1*. It thus becomes quite possible that in this experiment sufficient backflow to elevate pressures to 90 mm. could have occurred only when the coronary system was distended by considerably higher diastolic pressures (*circa* 45 mm.). This hypothetical condition is illustrated by the vertical line *b*.

Observations such as these suggest that, as far as the *anterior ramus descendens* territory of the dog is concerned, the systolic backflow is much less than generally believed. When a peripheral coronary vessel is perfused with Locke's solution at approximately their diastolic pressures (*circa* 20 mm.) jets of red blood can be seen to enter the cannula during each systole and to leave during each diastole. This phenomenon which attracts the attention of all workers leaves an exaggerated impression of backflow for it must be remembered that blood diffuses rapidly into adjacent saline and rarely shoots much beyond the tip of a cannula, the total capacity of which is only 0.1 cc. Experimental estimates by means of a flow recorder still to be described indicate an actual to and fro movement of about 0.04 cc.

SUMMARY AND CONCLUSIONS

In order to study the phasic changes in peripheral coronary resistance qualitatively and quantitatively, pressure changes in a peripheral coronary branch were recorded optically by two procedures.

Such records indicate that our current conceptions regarding the time relations, character and magnitude of peripheral coronary resistance require some revision:

1. Normally, the peripheral coronary pressure (P.C.P.) increases quickly during isometric contraction and the first moments of ejection, rises more gradually to a summit during the shortening phase, decreases abruptly during isometric relaxation and is influenced but little by subsequent lengthening of ventricular muscle.

2. Such time relations together with the demonstrations *a*, that P.C.P.

curves are not materially affected when the regions involved extend instead of shorten (ischemia), and *b*, that at constant diastolic pressures, systolic coronary pressure increases proportionately to systolic aortic pressure, when the latter rises, indicate that intramural and intraventricular tension rather than muscle fiber length predominantly determines the resistance.

3. The fact that the systolic maximum pressure is reduced somewhat when the involved muscle-area extends instead of shortens can be interpreted to mean either that muscle shortening is normally of supplementary assistance or that stretching increases the capacity of the coronary branches sufficiently to prevent full development of systolic pressure.

4. Peripheral coronary resistance is not affected to any discoverable extent by transmission of pressure from collateral branches because *a*, the magnitude of flow from an open peripheral ramus is very small; *b*, the steep and major rise of P.C.P. occurs prior to development of maximum aortic pressure, and *c*, clamping of the right or/and left circumflex rami produce no phasic changes in resistance and only such deviations in magnitude as can be better explained by concurrent changes in the dynamics of ventricular contraction.

5. In beats equivalent as regards contractile force, the systolic pressure maximum reached depends upon the diastolic pressure level from which a beat starts, i.e., upon the degree of coronary filling. Volume-elasticity studies of the coronary system, interpreted in conjunction with pressure changes and flow determinations, strongly suggest that the systolic backflow is of the order of 0.03 cc. which is considerably less than usually stated. Since this backflow is less than that required for development of the total pressure of which the myocardium is capable, pressure curves recorded from a peripheral ramus do not allow an appraisal of the maximum resistance developed under natural conditions of coronary distention.

The facts presented are of fundamental importance in understanding phasic changes of coronary flow and in interpreting the actions of drugs upon the intact coronary circulation.

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THE SO-CALLED NORMAL ALCOHOL OF THE BODY[1]

R. N. HARGER and ANNA L. GOSS

From the Department of Biochemistry and Pharmacology, Indiana University School of Medicine, Indianapolis, Indiana

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The statement has frequently been made that ethyl alcohol is a normal constituent of animal tissues and fluids. This idea was first advanced by Ford (1) in 1858. In the intervening seventy-seven years over a score of investigations upon this question have been recorded, practically all of which support Ford's conclusion regarding normal alcohol. In these investigations body fluids or tissues were distilled and these distillates, after more or less concentration and purification, were analyzed for "alcohol" by one of several methods. Reduction of dichromate was employed as the analytical procedure in thirteen of the studies (references 2 to 14); formation of acid after oxidation was used in three investigations (15), (16), (17); two groups (19), (20) employed the interferometer; and modifications of the Zeisel alkoxy method were used in two investigations (20), (21). None of these methods is specific for ethanol.

In the investigations cited most of the figures reported as alcohol range between one and ten milligrams per hundred grams of tissue or body fluid. Although these quantities are too small to have any connection with the problem of inebriety they are of the same order of magnitude as the normal figures for a number of body substances which are determined every day in clinical laboratories, and therefore, if correct, may be of biological significance and perhaps of clinical interest.

Only two papers report no "normal alcohol."[2] They are the studies of Lenoble and Daniel (7) and Kridelka and Bohet (11), using spinal fluid and blood respectively. However, these workers simply tried the Nicloux

[1] Part of this work was presented before a joint meeting of the sections on Biological Chemistry and Medicinal Chemistry of the American Chemical Society on April 1, 1931 at Indianapolis, Ind. and a preliminary report appeared in J. Indiana State Med. Assn. **25**: 384, 1932.

[2] Gettler, Niederl, and Benedetti-Pilcher (21) quote Umber (Ztschr. Klin. Med. **39**: 12, 1900), and Arnheim and Rosenbaum (Ztschr. Physiol. Chem. **40**: 220, 1904), as denying that alcohol is normally present in the body, but a perusal of the papers quoted fails to confirm this statement. These workers simply investigated the question whether or not blood or body tissues are capable of fermenting or consuming glucose in vitro, and they made no effort to study the question of "normal alcohol."

dichromate method using small quantities of material (2.5 to 5.0 cc.), which procedure would hardly allow the detection of concentrations of alcohol lower than about ten milligrams per hundred grams.

A consideration of the existing literature raises two questions:

1. *Is ethyl alcohol present in the concentrated distillates from body tissues and fluids?* Several of the studies describe the isolation of a material which supported combustion, reduced chromic acid with the formation of aldehyde and organic acid, and gave a positive iodoform reaction. J. Bechamp (15) reported the isolation of sodium acetate after oxidizing the alcohol-like substance, while Maignon (3) stated that he converted the acetic acid formed into ethyl acetate, ethyl butyrate, and cacodyl. Taylor (22) applied the Buchner test and recorded the formation of the ethyl ester of p-nitro benzoic acid. Using an ingenious micro distillation apparatus and other micro methods Gettler, Niederl, and Benedetti-Pilcher (21) reported the isolation from body tissues of an anhydrous fluid which boiled at around 78°C., having the carbon content of ethyl alcohol, and which was converted into ethyl iodide and ethyl benzoate. This evidence strongly points to the presence of ethyl alcohol in some of the concentrates obtained.

2. *Assuming that ethyl alcohol was found in some of these distillates was it present at the time of death?* The evidence on this point is by no means conclusive. The traces of alcohol reported might result from postmortem fermentation since the conversion of a small fraction of the tissue glucose to alcohol would account for the results obtained. Furthermore, the body contains several substances having ethoxy groups which might be hydrolyzed during the distillation or subsequent concentration.

We have attempted to approach this problem from a somewhat different angle than has been employed by previous investigators. The procedure adopted was suggested as a result of our observation that if the steam distillation of body tissues or fluids was prolonged beyond the point where all traces of pre-formed alcohol would be removed, *a volatile reducing substance continued to come over in the distillate in quantities almost as great as in the beginning.* This indicated that a part, at least, of the reducing substance obtained was either a material of low volatility, or the result of decomposition during the distillation. Accordingly, we steam-distilled specimens of tissues and body fluid collecting the distillate in two successive portions, each having a weight equal to that of the fluid or tissue used and after purifying and concentrating these distillates we determined their reducing power by a modified dichromate method. By adding alcohol (1 mgm. per 100 g.) in control experiments we showed that the added alcohol appeared almost quantitatively in the first fraction of the distillate. *Therefore, any reducing material appearing in the second fraction of the distillate could not be alcohol existing in the tissues before distillation. Since the first frac-*

tion must have contained at least as much of this material, which could not have been normal alcohol, as the second, the normal alcohol could not have been more than the difference between the reducing substance in fractions one and two. While the method employed is not specific for ethanol but simply determines volatile reducing substances secured by the procedure employed, it at least gives *maximum* figures for the amount of ethanol present. Although our studies have not settled the question regarding what fraction, if any, of the volatile reducing substances obtained is made up of ethanol, we believe that the results do contribute to a solution of the problem of "normal" alcohol in that they show that most of the figures reported by previous investigators are far too high.

Before analysis the distillates were purified and concentrated by fractionating first from acid and then from alkaline silver nitrate. To minimize the evolution of reducing substances less volatile than alcohol we distilled off only one-fifth of the volume in each fractionation. Control tests with small amounts of alcohol showed a recovery of about 87 per cent by this process. Besides resulting in lower figures for "alcohol" this procedure reduced the number of distillations to secure the desired concentration.

With urines and a few tissues we added a further purification step in which the final 10 cc. obtained by the process described above were refluxed with alkaline mercuric chloride, the procedure being a modification of the method of Gorr and Wagner (23) for the removal of acetone from ethanol.

Preliminary experiments confirmed the findings of Pringsheim (4) that there is a post-mortem increase in the volatile reducing substance in tissues. Consequently, we made an effort to steam-distill the material as soon as possible after the death of the animal, or removal of blood or urine from living subjects. Since distilled water on standing develops a small amount of volatile reducing substance, only freshly distilled water was used, and in generating steam the first portion of the steam was discarded.

Procedure. One hundred grams of urine, blood, or hashed tissue were placed in a flask and to this were added 100 cc. of freshly distilled water, 0.5 gram of tartaric acid, and a small piece of paraffin. The flask was heated in boiling water and its contents steam distilled in the usual manner, the vapor evolved passing to the condenser through a column containing a Kjeldahl connecting bulb. The distillate was collected in two successive 100 cc. portions. In a few cases a third or fourth 100 cc. fraction was collected. Each 100 cc. portion was then transferred to an ordinary 250 cc. distilling flask and acidified with 0.2 cc. of concentrated sulfuric acid. The flask was connected to a small vertical condenser, and its contents boiled over a free flame until exactly 20 cc. of distillate had been collected. This distillate was then transferred to a 100 cc. distilling flask and two 10 cc. portions of distilled water used to rinse out the condenser

and receiving flask, these washings being added to the 20 cc. of distillate. Three cubic centimeters of 5 per cent silver nitrate were next added, followed by 2.5 cc. of 10 per cent sodium hydroxide. This flask was connected with the same type of condenser used in the first distillation and the contents (45.5 cc.) boiled over a free flame until exactly 9 cc. of distillate were collected. Any alcohol adhering to the condenser tube was rinsed out with two 0.5 cc. portions of distilled water. The resulting 10 cc. thus represented a ten-fold concentration of the original steam distillate. After mixing, 5 cc. were withdrawn and analyzed for reducing power by a modified dichromate method described elsewhere (24). In the case of human blood, 50 cc. samples were employed, the volume of distillates and reagents being reduced to one half, and the final distillate of 5 cc. was all used for the determination of reducing material. With urine and a few tissues the final 10 cc. obtained as outlined above were transferred to a flask to which were then added 5 cc. of 5 per cent mercuric chloride, 3 cc. of 3 per cent sodium hydroxide and two 1 cc. portions of water, the last being used to rinse out the receiving tube. The flask was connected to a reflux condenser by means of a ground glass joint and the contents boiled for 15 minutes. At the end of this time the flask was cooled and the condenser tube rinsed out with 2 or 3 cc. of water. The flask was then connected to a condenser for distillation and boiled until exactly 10 cc. of distillate had collected; 5 cc. of this distillate were then analyzed for reducing power and the remainder of the distillate tested for acetone by the method of Behre and Benedict (25). In all cases no acetone was found although it was always present in the concentrates from urines not treated with alkaline mercuric chloride. Control experiments showed that this refluxing with alkaline mercuric chloride served to remove acetone without destroying ethanol, whereas refluxing with alkaline silver nitrate failed to remove acetone. Before each distillation the condenser tubes and distilling flasks were cleaned by being boiled in concentrated nitric acid after which they were carefully rinsed with distilled water. This was done because a volatile lipoid-like material frequently was deposited in the condenser tubes during concentration of the tissue distillates.

RESULTS. 1. *Reducing material in successive portions of distillate.* Hundred gram samples of human brain or beef liver were steam-distilled as described above, several portions of distillate being collected. Five cubic centimeter specimens of these distillates were analyzed directly for reducing material, and the remaining 95 cc. portions were concentrated tenfold as described, and then analyzed. The results are given in table 1. It will be noted that the presence of reducing material in the distillates persisted long after all pre-formed alcohol should have been removed, and that the concentration process removed a great deal of the reducing material.

2. *Recovery of added alcohol.* We first determined the amount of alcohol recovered in the concentration procedure. One milligram of alcohol was added to 100 cc. of freshly distilled water and this was concentrated in the two steps, as described, first from acid and then from alkaline silver nitrate. In six experiments analysis of the final concentrates of 10 cc. showed a recovery of 0.894, 0.874, 0.890, 0.874, 0.824, and 0.844 mgm., respectively, or an average of 0.867 mgm. Thus an average loss of 13.3 per cent occurred during concentration.

We next tried adding 1 mgm. of alcohol to 100 grams of hashed beef liver or animal blood[3] and determined the amount of alcohol recovered when these tissues were steam distilled, concentrated and purified in the manner described. Controls on the same tissues were run simultaneously.

TABLE 1

Reducing substance from successive fractions of distillate

TISSUE	FRACTION OF DISTILLATE	DICHROMATE CONSUMED BY DISTILLATE FROM 100 GRAMS OF TISSUE*	
		Analyzed directly	Purified and concentrated
		cc. of 0.0434 N $K_2Cr_2O_7$	*cc. of 0.0434 N* $K_2Cr_2O_7$
Human brain	First 100 cc.	1.71	0.72
Human brain	Second 100 cc.	0.69	0.334
Human brain	Third 100 cc.	0.55	0.226
Beef liver	First 100 cc.	2.72	0.948
Beef liver	Second 100 cc.	2.14	0.440
Beef liver	Third 100 cc. (rapid distillation)	2.72	
Beef liver	Fourth 100 cc. (very rapid distillation)	7.50	

* One cubic centimeter of 0.0434 N dichromate is consumed by 0.5 mgm. ethyl alcohol.

In four experiments the figures for the recovery of alcohol in the first 100 cc. of distillate were respectively[4] 0.998, 0.923, 0.836 and 0.883 mgm. or an average of 91.0 per cent. The figures for alcohol recovered in the second 100 cc. of distillate were respectively[4] 0.01, 0.20, 0.02 and 0.00 mgm. of alcohol or an average of 5.8 per cent. When alcohol was added and the material allowed to stand for two hours before being distilled, the yield of alcohol was[4] 71.2 and 61.5 per cent in the first 100 cc. and 3.6 and 16.4 per cent in the second 100 cc. Ford (1) and Fleischman and Trevani (26) noted that blood is able to decompose alcohol in vitro. The addition of but one milligram of alcohol per hundred grams of blood or tissue caused

[3] The blood was preserved with 1 per cent of KF, 2 H_2O.

[4] Corrected for a loss of 13.3 per cent during concentrations.

a great rise in the reducing material appearing in the first 100 cc. of the distillates, the increase ranging from almost threefold to elevenfold.

3. *Effect of delay before analysis.* Hog livers were removed from the animals and immediately brought to the laboratory. The tissue was hashed and one hundred gram portion was steam distilled as quickly as possible. The remainder was placed in a closed vessel in a well cooled refrigerator and after intervals of twenty-four hours and six days hundred gram portions were steam-distilled. Prior to analysis the steam-distillates were purified and concentrated ten-fold as usual. The consumption of 0.0434 N. dichromate by the first 100 cc. portion of distillate was as follows: liver A, one-half hour, 1.075; twenty-four hours, 1.688; six days, 20.80; liver B two hours, 0.828; twenty-four hours, 1.910; six days, 53.32. Delay before distillation caused very little change in the reducing material appearing in the second 100 cc. of distillate.

4. *Reducing material obtained upon prompt distillation of fresh body tissues and fluids.* In these experiments special precautions were taken to steam distil the material as quickly as possible after the death of the animal or the removal of blood or urine from living subjects. In each case a 100 gram sample was steam distilled except with the human bloods where a 50 gram sample was used. The two fractions of distillate obtained from each sample were then concentrated and purified as described above under *procedure*. In the case of human urines and also with certain tissues the distillate from the alkaline silver nitrate treatment was further purified by refluxing with alkaline mercuric chloride as described above. A large number of tissues, bloods and urines were put through this procedure. Table 2 records typical results from these experiments. In order to save space we have included only about half of the results since they were fairly uniform. The results given include the maximum variations found.

Discussion. While it is true that the method of analysis employed in this study is not specific for ethanol, the same criticism applies to all other methods for estimating ethanol, of which the authors are aware. Theoretically it might seem that greater accuracy would be attained by estimating ethanol as a conversion product such as the iodide or acetic acid. However, the much higher figures reported elsewhere when these methods were applied to this problem would indicate that reactive substances other than ethanol were present.

It might be argued that the continued evolution of reducing material when the steam-distillation is prolonged may mean that traces of normal alcohol are tenaciously held by the tissues and therefore not all removed in the first fraction. This theory would appear to be disproved by our experiments on the recovery of added alcohol. Here, after adding only one milligram of alcohol to 100 grams of body tissue, we found that practically all of the added alcohol appeared in the first fraction of the distillate.

Even when the alcohol was in contact with the tissue (blood) for two hours there was very little increase in the amount of reducing material in the second fraction of distillate.

TABLE 2

Reducing material in successive distillates from body tissues and fluids

	Dichromate consumed by distillate from 100 grams of tissue or fluid*			Maximum figure for normal alcohol (a-b) x 0.5 x 1/0.867†
	First 100 cc. of distillate (a)	Second 100 cc. of distillate (b)	Difference (a-b)	
	cc. of 0.0434 N $K_2Cr_2O_7$	*cc. of 0.0434 N* $K_2Cr_2O_7$	*cc. of 0.0434 N* $K_2Cr_2O_7$	*mgm. per 100 grams*
Dog A:				
Blood	0.236	0.220	0.016	0.009
Liver	0.422	0.218	0.204	0.199
Kidney	0.366	0.290	0.076	0.044
Brain	0.506	0.422	0.084	0.049
Muscle	0.392	0.328	0.064	0.037
Dog B:				
Blood	0.200	0.267	None	None
Brain	0.734	0.530	0.204	0.119
Liver	0.760	0.370	0.390	0.227
Kidney	0.377	0.134	0.243	0.142
Muscle	0.540	0.150	0.390	0.227
Human blood, subject:				
H. W. B	0.167	0.160	0.007	0.004
R. N. H	0.180	0.173	0.007	0.004
D. J. W	0.217	0.293	None	None
H. R. H	0.347	0.300	0.047	0.027
Beef liver‡	0.272	0.103	0.169	0.085
Beef kidney‡	0.179	0.075	0.104	0.060
Human urines:‡				
Specimen 1	0.385	0.281	0.104	0.060
Specimen 2	0.308	0.127	0.181	0.104
Specimen 3	0.380	0.262	0.118	0.068
Specimen 4	0.498	0.177	0.321	0.185
Specimen 5	0.342	0.134	0.208	0.120
Specimen 6	0.241	0.098	0.143	0.083

* One cubic centimeter of 0.0434 N dichromate is consumed by 0.5 mgm. ethyl alcohol.

† Corrected for loss of 13.3 per cent of alcohol during concentration.

‡ Refluxed with alkaline mercuric chloride.

Our assumption that the maximum figure for pre-formed alcohol is represented by the excess of reducing material in the first fraction of distillate over that in the second fraction probably errs in the positive direction,

because the third fraction contained somewhat less reducing material than the second, indicating that still more of the reducing material of the first fraction was not due to pre-formed alcohol.

Finally, our conclusion that the normal alcohol, if any, is much smaller than the figures usually given is supported by two large scale experiments reported in the literature. Ford (1) distilled fifty pounds of ox lung and obtained no alcohol. He attributed this negative result to the fact that the experiment was done during hot weather. In the large scale experiment recorded by Gettler, Niederl, and Benedetti-Pilcher (21), 28 kilos of pigs' brains were employed and the figure obtained for "alcohol" was only 0.07 mgm. per 100 grams. This very low figure is in marked contrast to the other figures reported by these investigators using smaller quantities of tissues. Thus their maximum figure for human liver "alcohol" is *eighty times greater* than this figure for pigs' brain!

SUMMARY AND CONCLUSIONS

1. Fresh specimens of urine, blood, and body tissues were promptly steam-distilled, two or more successive fractions of distillate being collected, each fraction having a weight equal to that of the specimen used. Each fraction of distillate was then purified and concentrated ten-fold, and these concentrates tested for reducing power by a micro dichromate method.

2. In all cases the first fraction of distillate contained a small amount of reducing material, which, expressed as ethanol, ranged from 0.09 to 0.38 mgm. per 100 grams of body tissue of fluid.

3. These small quantities of reducing material in the first fraction of distillate were shown to be largely not pre-formed ethanol because succeeding fractions of the distillate contained almost as much reducing material.

4. In control experiments it was shown that a trace of added ethanol (1 mgm. added to 100 g. of body tissue or fluid) was almost all recovered in the first fraction of the distillate.

5. Since the reducing material in the second fraction of the distillate could not be pre-formed alcohol, and since the first fraction of distillate must have contained at least as much of this reducing material, which also was not normal alcohol, then the maximum figure for normal alcohol may be represented as A-B where A and B represent the quantities of reducing material in the first and second distillate fractions respectively.

6. Our results with fresh tissue, calculated on the basis just described, give maximum figures for normal alcohol, expressed as milligrams of ethanol per 100 grams of body tissue or fluid, as follows: blood 0.0 to 0.027, brain 0.049 to 0.119, liver 0.085 to 0.227, kidney 0.044 to 0.142, muscle 0.037 to 0.227, and urine 0.060 to 0.185.

7. When body tissues were allowed to remain in a refrigerator for some time before being steam-distilled, an increase was noted in the quantity of

reducing substances appearing in the first fraction of distillate. Thus, after twenty-four hours the quantity of reducing substance was almost doubled, and after a delay of six days the increase was more than twenty-fold.

8. The normal concentration of body ethanol, if any, is very much smaller than has been reported by most previous investigators.

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RATES OF RESORPTION IN THE GALL BLADDER

Further Experiments with Methylene Blue on Rabbits

BÉLA HALPERT, PAUL A. O'CONNOR and WILLIAM R. THOMPSON

From the Department of Pathology, Yale University School of Medicine

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In former experiments with methylene blue on rabbits it was shown that it is possible to calculate the mean rate of flow of bile through the cystic duct and the amount of fluid resorbed from the gall bladder per unit of time (1). It was observed that when the bile was secreted against a constant hydrostatic pressure of 50 mm. greater than that of the atmosphere, the gall bladder resorbed approximately half the volume of its contents per hour. The present studies were designed to determine whether the rates of resorption from the gall bladder are influenced by pressure. Thus experiments were planned in which bile was secreted against a constant hydrostatic pressure of 0, 75 and 100 mm., respectively, greater than that of the atmosphere.

Method. The method used was identical with that applied by Halpert, Thompson and Marting in their experiments (1). With the animal under ether anesthesia, a glass cannula was tied into the common bile duct close to its termination in the duodenum. The cannula was joined with a rubber tube to the inlet of a glass trap connected to a remote gas chamber with a hydrostatic pressure maintained at the desired level. After the proper adjustments were made, the bile was carefully drained from the trap and discarded. Collections were then commenced over successive intervals of one-half hour. Immediately after the first of these was started 2 ml. of a 1 per cent solution of methylene blue per kilogram of body weight of the animal were injected into the marginal vein of the left ear. The volume of bile in each half hour collection was measured and its methylene blue content determined. The experiments were concluded at the end of three hours, at which time the entire content of the gall bladder was removed and its volume and methylene blue concentration determined (2).

As in former experiments (1) the calculations were based on the assumption that in any given interval during the experiment the volume of bile which entered the cystic duct was approximately proportional to that which left the common bile duct, and that the amount of bile withdrawn from the common bile duct was replaced by an equal volume of bile of the

same methylene blue concentration as that entering the cystic duct. However, essentially the same results are obtained from an alternative assumption that the rate of flow through the cystic duct is independent of the rate of flow through the common bile duct.

EXPERIMENTAL DATA. The pertinent data of the experiments are summarized in table 1. It may be seen that there are considerable individual

TABLE 1

The transportation of methylene blue in the bile of the rabbit following intravenous administration of the dye (2 ml. of a 1 per cent solution per kilogram of body weight)

RABBIT (MALES)		HYDROSTATIC PRESSURE	VOLUME OF BILE OBTAINED FROM		TOTAL METHYLENE BLUE IN BILE FROM		MEAN RATE OF FLOW OF BILE THROUGH THE CYSTIC DUCT (r)	$\frac{r}{v_0}$
Number	Weight		D. choledochus (V)	Gall bladder (v_0)	D. choledochus (M)	Gall bladder (m)		
	kgm.	*mm.*	*ml.*	*ml.*	*mgm.*	*mgm.*	*ml./hr.*	*hr.*$^{-1}$
2	2.5	0	19.6	1.0	5.42	0.633	0.746	0.762
4	3.1	0	50.4	1.7	12.67	0.155	0.213	0.121
5	2.9	0	31.9	1.3	11.33	1.22	1.130	0.818
6	3.2	0	24.4	2.8	9.01	1.10	1.004	0.355
Mean			31.6	1.7	9.61	0.78	0.77	0.51
a.d.			9.6	0.6	2.39	0.38	0.29	0.28
7	2.6	75	33.8	3.9	8.37	2.50	2.15	0.516
8	2.70	75	29.0	2.1	11.20	0.656	0.581	0.270
9	2.97	75	29.7	1.5	7.91	0.694	0.904	0.580
11	2.97	75	48.4	2.0	2.53	0.770	0.511	0.244
Mean			35.2	2.4	7.50	1.16	1.04	0.40
a.d.			6.6	0.8	2.49	0.48	0.56	0.15
12	2.85	100	27.7	2.7	7.004	1.125	1.44	0.548
14	2.80	100	29.7	3.7	5.383	0.748	1.38	0.372
15	3.1	100	38.0	3.1	8.237	0.896	1.39	0.444
17	3.0	100	14.3	1.9	6.180	1.9	1.34	0.771
Mean			27.4	2.8	6.70	1.17	1.39	0.53
a.d.			6.6	0.6	0.92	0.80	0.03	0.13

variations in the volume (V) and the methylene blue content (M) of the bile obtained from the ductus choledochus during the experimental period. Similar individual variations were noted in the volume (v_0) and the methylene blue content (m) of the bile obtained from the gall bladder. The volume of bile and its methylene blue concentration in the gall bladder as well as the mean rate of flow of bile through the cystic duct (r) appeared to be somewhat greater in the experiments with higher pressure. How-

ever, the relative rates of resorption from the gall bladder $\left(\frac{r}{v_0}\right)$ were about the same in the experiments at 0, 75, and 100 mm. of hydrostatic pressure, i.e., approximately one-half the volume of the contents of the viscus per hour.

SUMMARY

Methylene blue was given intravenously to rabbits and the bile, secreted against a constant hydrostatic pressure respectively 0, 75 and 100 mm. above the atmosphere, was collected from the common bile duct and the contents of the gall bladder were removed at the end of the experiment. The methylene blue content of each sample of bile was then determined and the amount of bile which entered the gall bladder during the experimental period was estimated. The data thus far obtained indicate that the rate of resorption of fluid from the gall bladder was greater in the experiments with higher pressure. However, the ratios of these rates to the volumes of gall bladder contents were about the same, approximately half the volume of the contents of the viscus per hour.

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THE WATER AND CHLORIDE EXCRETION OF DECEREBRATE CATS

MARGARET SUMWALT, W. H. ERB AND H. C. BAZETT

From the Department of Physiology, University of Pennsylvania Medical School

Received for publication April 10, 1935

That diabetes insipidus may be experimentally induced by damage to the hypothalamus or hypophysis is now generally recognized. (For a short review, see Leschke, 1933.) So is the conception that the control over water balance normally exerted from this region is humoral, rather than nervous. Yet conflicts of evidence occur, even in connection with these well established points; and other details, scarcely less important for visualizing the mechanisms concerned, remain open questions. For example, irritation rather than deprivation of nervous structures is occasionally advocated as the cause of diabetes insipidus; and sometimes polyuria is held responsible for polydipsia, sometimes the reverse.

The study to be reported in the present paper utilized familiar techniques in a combination which, for this field, is somewhat novel. Neglecting the localization problem, we treated the pituitary body with neighboring structures as a unit, and sought to identify the nature of their joint influence upon chloride and water excretion in cats by comparing the effects of three types of operation. The pituitary and hypothalamus were removed from the cranium entirely; or their nervous connections with the rest of the animal were severed with as little disturbance as possible to their blood supply; or they remained *in situ* with nervous and also possible chemical pathways intact. For more complete regulation of the environment, activity, diet, and urine collection of the cats, all of the lesions were studied against a background of decerebration.

Method. Our study is based upon 46 operated animals. Three were acute preparations, decerebrated under ether by the guillotine, without aseptic precautions. Their urine was collected through a bladder cannula each half-hour after the operation. The other 43 cats were maintained in a living condition for from 3 to 16 days, while their daily water and chloride excretion were measured for comparison with their daily intake. The three types of operation already outlined divided them into three groups. The lesions will be described with their results. All shared alike in the general treatment which they received. The operative technique described by Bazett and Penfield (1922) was used; except that, for chloroform, dial

combined with nembutal was substituted in the proportions suggested by Bazett, Alpers, and Erb (1933); and the cranial dead space was not invariably filled with wax.

Most of the chronic cats underwent, immediately after the brain operation, a suprapubic cannulation of the urethra with a glass cannula which penetrated the internal sphincter, so that drainage was continuous. (In a few cases not so cannulated, feces and urine were collected together by a funnel, and analyzed together.)

A rubber tube on the urethral cannula conducted urine by gravity flow into a flask. We made no attempt to prevent evaporation or to allow for it, but relied upon the high humidity of the room to keep this loss insignificant. The accumulated 24-hour specimen was collected each morning and measured. Samples were analyzed by the modified Volhard-Harvey titration for urinary chlorides as described by Peters and Van Slyke (1932). It was almost invariably necessary, with the urine of both normal and decerebrate cats, to digest the sample with potassium permanganate over a flame, before the mixture of urine with nitric acid and silver nitrate would be sufficiently colorless for titration.

Previous to operation no attempt was made to regulate the diet of the cats. Their postoperative regime consisted of a daily 200 cc. of milk, which was found to contain 360 mgm. of NaCl if all the chloride content is assumed to have been in this form. This was administered in two doses, morning and evening. The actual daily intake of an animal was sometimes less than this, when his resistance to feeding made it seem wiser to withhold a portion of a meal, or part was lost by vomiting. More than this they received only in a few instances, when we doubled the water content or the salt content of a feeding or two, with several cats, to see whether a change in the fluid/salt ratio of the diet would alter the type of excretion. Since these sporadic experiments slightly mar the uniformity of regime, the number of cat-days upon which such special rations were given appears in table 1. The number was small relative to the total number of experimental days; and no effect which could be attributed to the special feedings was ever observed to follow them. (For instance, the volume of urine excreted increased as often as it decreased, during a period in which extra water was given.) Hence the results are discussed as though the diet had been uniform. The other features of postoperative care, such as temperature regulation and recording, humidification, and cleaning, followed closely the method described by Bazett, Alpers and Erb (1933).

Our comparison of ingested water and chloride with urinary water and chloride does not pose as a study of water and chloride balance complete. We never recovered from any cat, over a period of several days, as much as he received in that same time (though occasionally, for one or two days, water output exceeded intake). At least half of the ingested fluid was

probably lost by evaporation.[1] When the excreta were collected together, fecal admixture did not appear to increase either volume measurements or chloride measurements significantly. Some chloride may have been lost in the nasal secretions, and by vomiting, in a few cats. At any rate, unmeasured losses must have been roughly the same for all the cats (with one group exception to be mentioned later), and variations among them therefore reflect real differences in their water or chloride balance.

We performed gross but not microscopical autopsies. When the animals died unexpectedly, as they usually did, the incubation which ensued before they were discovered often made the gross autopsies unsatisfactory. The animals are therefore classified according to the intention of the operation, except when autopsy or physiological behavior other than water balance revealed a failure.

There is no definitely known reason why decerebrate cats of any of our three types should not survive for long periods. We made every effort to prolong their lives. The commonest manifest reasons for death were accidents to the heat regulating system, aspiration of vomitus, or infection (cranial or pulmonary). The causes of about 25 per cent of the fatalities were obscure. Despite the diversity of deaths that befell, success in prolonging survival seemed to be curiously correlated with the type of chloride excretion, and this in turn with the kind of section. This suggests that the nature of the lesion may have been responsible for some of the inexplicable deaths and even perhaps indirectly for some of those in which the immediate cause seemed to be apparent. Susceptibility to infection or frequency of vomiting, for instance, may have depended remotely on chloride unbalance.

It is difficult to subject the normal cat to the conditions borne by the decerebrates. Three kinds of approximation to normal controls were studied, and are shown in table 1. One normal cat which was offered the standard diet for four days in a cage, though not in the incubated room, excreted a daily average of 60 cc. of urine with a content of 210 mgm. of NaCl. Another normal cat, kept free in the laboratory, and trained to micturate in a glass crystallizing dish, ate the diet of the experimental animals for two four day periods, with results which closely resembled those with the caged cat. His daily excretion during one of these periods is shown in figure 2 A. During another four day period he was given water *ad lib.*, besides the 200 cc. of milk, in an attempt to compensate him for his greater loss by evaporation as compared with the humidified cats.

[1] Several animals of the "island" type, in which the chloride excretion was unfortunately not being investigated, were sufficiently normal to survive over three weeks, and showed little or no change in weight. In these the volume of fluid excreted averaged half of that ingested. Though the humidity of the room was 80 per cent at 26°, considerable amounts of water could be evaporated at body temperature.

Under these conditions his average daily water and chloride excretion was greater than before: 110 cc. with 325 mgm. As an attempt at a third type of control, a cat was cannulated and kept anesthetized with dial under the same conditions as the decerebrate animals. It survived two and a half days, and yielded figures which do happen to agree fairly well with the caged and free cats'. It could not be considered a normal animal, however.

Results. *Group 1. High section.* Cats in which both nervous and humoral pathways from the pituitary and hypothalamus were to be left

TABLE 1

Group figures for the survival time, chloride excretion, and water excretion of control and decerebrate cats

Their intake was 200 cc. of milk daily, containing 360 mgm. of NaCl, except on a few days when special rations described in the text were given. The number of days upon which the averages for excretion are based are given in adjacent columns. Chloride is given as NaCl.

GROUP	NO. OF ANIMALS	NO. OF DAYS SURVIVAL	NO. OF DAYS OF SPECIAL RATION	NO. OF DAYS	MEAN VOL. PER DAY	NO. OF DAYS	MEAN NaCl PER DAY	NO. OF DAYS	MEAN CONC. NaCl
					cc.		mgm.		per cent
Control:									
Caged cat and trained cat....	2			10	62	9	264	9	0.426
Trained cat with water ad lib..	1			3	110	3	325	3	0.295
Dial cat (abnormal)..........	1	2		2	43	2	159	2	0.37
Operated:									
Group 1. High section.......	4	14	4	14	45	14	76	14	0.169
Group 2. Low section........	9	33	9	30	131	29	97	29	0.074
Group 3. Island section......	30	179	9	155	97	131	129	131	0.133
Long lived...............	13	123	4	102	98	85	154	85	0.157
Short lived................	17	56	5	53	95	46	82	46	0.086
Small volume............	8	23	0	20	51	17	68	17	0.133
Large volume............	9	33	5	33	115	29	91	29	0.050

intact were prepared by a single nearly horizontal section (level 1, fig. 1), which sloped from a point just in front of the posterior colliculi dorsally to the optic chiasm ventrally. Structures above this level were removed. These cats developed at least partial temperature control about two days after operation. They responded to loud sounds, and sometimes violently resisted feeding, cleaning, and weighing. Though tied to their beds, they performed running movements for long periods, punctuated with intervals of complete quiet. Since they suffered the smallest operative damage of any group, they might be expected to resemble normal cats most closely,

and so to constitute an operated control. The result proved otherwise. They differed greatly from the normal cats in two ways: survival less than five days, and a chloride output averaging 76 mgm. as against 264 mgm. in the normal animals. Their water excretion also was lower than normal, though the difference in this respect is less striking.

The results in this group, however, were undoubtedly complicated by the excessive activity of the animals. For instance, dehydration through overventilation must have reduced the available water for excretion. Perhaps the diminutive urine volume, in turn, was responsible for the low chloride excretion; though the normal cats, excreting only 38 per cent more water, eliminated 250 per cent more chloride. Group 1, therefore, failed to furnish preparations which are comparable with the other two groups.

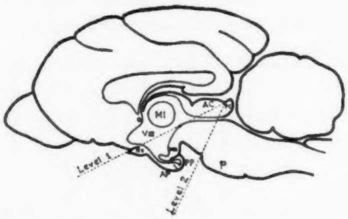

Fig. 1. The two levels of section which were used in decerebrating, shown diagrammatically in relation to the structures which lie in or near the median plane of the cat's brain.

AC—anterior colliculus; *PC*—posterior colliculus; *MI*—massa intermedia; V_{III}—third ventricle; *N2*—optic nerve; *MB*—mammillary body; *P*—pons; *AP*—anterior lobe of pituitary; *PP*—posterior lobe of pituitary.

Group 2. Low section. To ablate the pituitary and hypothalamus, the spatula passed from the same point dorsally as level 1, in a more nearly vertical plane downward to the anterior edge of the pons (level 2, fig. 1), and removed everything anterior to that level. The nine cats prepared in this way resembled group 1 in shortness of life and low rate of chloride excretion. Unlike group 1, they excreted a large volume of water. The average, 131 cc., exceeded the average in any other operated group; and surpassed the control averages, even in that experiment upon a normal cat when water was allowed freely in addition to the standard diet. But average figures do not do full justice to the peaks of water output which they achieved on single days: 260, 250, 230, 220, 200, 200, 190, 160, 40 cc. Four of the nine animals excreted more than 200 cc. a day for two days. Such a rate was never maintained for more than two days. It is not to be expected that on a fixed intake of 200 cc., with simultaneous losses through other channels, a cat could continue to excrete so large a volume. These animals lost weight more rapidly than the other groups. With four animals, the peak excretion occurred during the first or second day, followed

by steady subsidence (fig. 2 C); in another four, it occurred during the third day preceded by considerably smaller volumes (fig. 2 D), and followed by subsidence if they survived through a fourth day. One animal differed from the others in not exceeding 40 cc. for any day of its survival.

We were interested in the rapid onset of polyuria which occurred in four of these cats. The three acute preparations which have been mentioned were therefore studied in this connection. The guillotine method was used in order to obviate all suspicion that any pituitary fragments

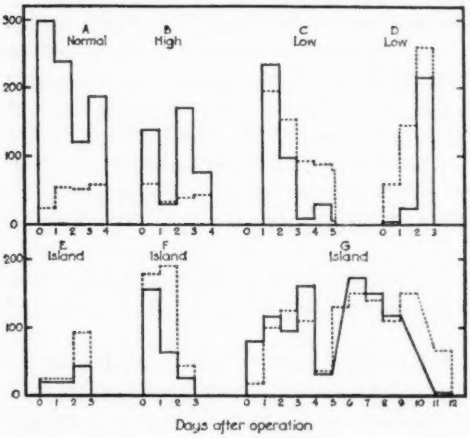

Fig. 2

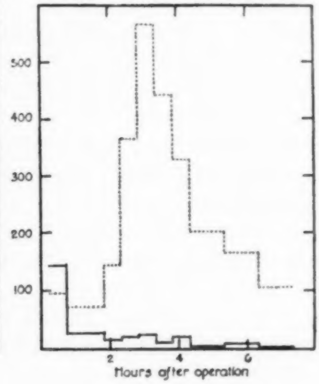

Fig. 3

Fig. 2. Sample individual records, showing types of case histories and the sort of daily variations encountered in a single case. Death occurred on the day following the last collection shown in each case. Solid line = NaCl excretion in milligrams per day. Dotted line = H_2O excretion in cubic centimeters per day.

Fig. 3. Sample record from an acute decerebrate. Urine was collected every half hour, but the units are for daily rates calculated on the basis of these collections. Solid line = milligrams of NaCl. Dotted line = cubic centimeters of H_2O.

remained. Ether was discontinued as soon as decerebration was performed. Within two hours thereafter in all three cats, the chloride rate had fallen considerably. At the end of six hours it was vanishingly small. Meanwhile the volume rate of urine excretion rapidly increased. (See fig. 3 for a sample record, in which the units chosen to express rate are the same as for the chronic cats.) The acute animals were killed at the end of eight or nine hours. Two of the three, by this time, were showing a falling rate of water excretion, and one of them an increasing chloride rate. They had received no food or fluid, either during the experiment, or in the twelve hours preceding it.

The operation undergone by the animals of group 2 was expected to

determine whether ablation of the pituitary and neighboring regions was capable of giving rise to an experimental diabetes insipidus, when there could be no question of irritation to surviving tissues. The three acute cats and eight out of the nine chronic cats did develop polyuria. It was easy to ascertain, either at the time of operation, or at autopsy, that the hypothalamus and all other parts of the brain overlying the sella turcica, down to the pons, had been completely removed. Therefore irritation of these could not have caused the polyuria. Hemorrhage or other damage at the level of section might conceivably irritate tracts or centers in the remaining brain stem; but no evidence has ever been presented which tended to show that stimulation at this level leads to disturbance of water metabolism. It is possible that fragments of the pituitary remained in the sella of the chronic cats. In two of them, in fact, tissue was found there at autopsy, though it was not histologically identified. But certainly in the acute animals no trace of any of the pituitary or hypothalamus remained. The chronic and acute animals taken together seem to indicate that the simple removal of hypophysis and hypothalamus can cause diabetes insipidus.

There can be no doubt that in the present experiments polyuria, when it appeared, was primary, because the animals' daily intake was limited to 200 cc. of milk.

The low chloride output of the group 2 cats, though inexplicable, requires comment. In eight of the nine cases, the trends of water and chloride excretion were roughly parallel to each other, as they are in graphs C and D of figure 2. Nevertheless, water and chloride excretion seem to be independent quantities, since this group, which surpassed all others in water output, eliminated an average of only 97 mgm. NaCl per day at a concentration usually below 0.1 per cent. Indeed the low average of chloride output combined with the unusually high volume output must imply an increasing chloride concentration within the animals. Such an accumulation might be sufficient reason for their short survival. We attempted to test the inference of accumulation by analysis of blood samples, but too few samples were obtained at the desired times to substantiate the point. Failure to eliminate chloride may have been due to diminished ability of the kidney to excrete chloride. However, in cases where chloride output improved progressively (for instance, the case of fig. 2 D), a corresponding improvement in kidney function is not necessarily indicated, since the blood chloride level was presumably increasing.

The term "decerebrate" is often used, as we have used it, in a broad sense which covers a rather wide variety of sections. Probably the commonest preparation described by this term corresponds closely to our animals of group 2. The point seems worth noting, since "decerebrate" animals have been used in several studies of water diuresis. Unless their

background of natural urine excretion is also studied, and found normal, it is not safe to extend conclusions obtained from such preparations to intact animals.

Group 3. Island section. The pituitary and the hypothalamus in the animals of group 3 were isolated nervously from the rest of the body by a vertical transection at level 2; but they were left in place with their blood supply as little damaged as possible. In eight cats the whole severed brain was left in place after the method described by Keller (1932). In the remaining twenty-two the first section, at level 2, was followed by another above it at level 1, and everything anterior to level 1 was removed (Bazett and Penfield, 1922). For our purpose, the latter method was better adapted: because, though the upper section may sometimes have invaded hypothalamic territory, or have wrought more disturbance to the circulation than Keller's method, it made the crucial lower section far easier to complete with certainty.

Both group 3 with this island section operation, and group 1 were intended to show whether the presence of the pituitary and hypothalamus could prevent polyuria. The island section was expected, besides, to test the humoral theory. If the polyuria which developed in eight out of nine cases in group 2 failed to put in its appearance in group 3, it would furnish strong evidence for the chemical nature of the influence upon water balance.

Actually, the volume output of island section cats averaged 97 cc. compared with 131 cc. for group 2. Individual figures give the same impression as group figures, that water excretion tends to be lower when the disconnected pituitary and hypothalamus are left in. The mean difference is rather large when one considers that limited intake must have levelled down the output of the animals with diabetes insipidus. Yet the variations, too, are large for a group which embraces only thirty individuals.

Two operative methods had been used in the preparation of group 3, but this fact does not explain the variability, since the results of both were equally divergent. Water excretion was completely independent of chloride excretion and of length of life. However, the group seemed to split quite sharply into two subgroups on the basis of survival time; or somewhat less clearly, on the basis of chloride output. These two factors ran roughly parallel. (See fig. 4.) Seventeen cats died in less than five days, like the animals of groups 1 and 2, and excreted 82 mgm. of NaCl per day. The rest lived an average of nine days and excreted 154 mgm. per day. The latter output, though still far from equalling their intake, is nearer normal than the output of either of the other groups. The long lived animals, moreover, appeared to be in water balance, since their weight losses were small, and their volume excretion lay between that of the control with water *ad lib.* and the controls on rigidly limited diet. In short, the island

section produced thirteen cats which were more stable and more nearly normal in excretion than any others of the operated cats. (See fig. 2 G, for an example.)

But it failed to do the like for the other seventeen. They are characterized in general by brief survival and a meagre chloride output. A few excreted chloride well; but at least a fraction of these may have been cats

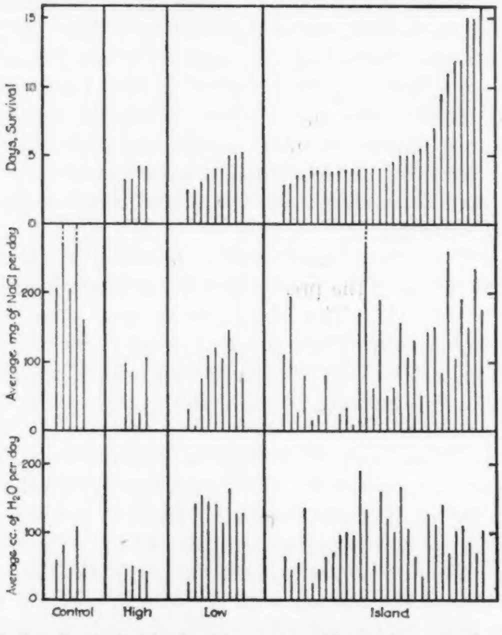

Fig. 4. Graph showing individual performance of both the controls and the chronic decerebrate animals. Ordinates are survival, average chloride, and average water output of single cats. Abscissae are the cats' numbers when they are arranged in order of increasing survival. Notice especially the clear division of group 3 into short and long survivals; and that a low chloride output usually corresponds to an incapacity to survive, but that water output bears no systematic relation either to survival or to chloride output. The limits of individual variation, though not of daily variation, appear in this graph.

destined to live long, and killed by mechanical accident. They fall naturally into still further subdivision on the basis of water excretion. Nine of them conveyed away their small output of salt in a small volume (fig. 2 E), as did group 1, but without any excessive muscular activity. The others approached the diabetes insipidus type of excretion, averaging around 100 cc. per day (fig. 2 F), and attaining, for one day each, the following maxima: 260, 260, 230, 190, 170, 160, 130, 130, 130 cc.

In group 3 the results, superficially considered, favor the humoral theory. Analyzed they are less convincing, but still suggestive in the same direction. The weak points in the evidence are two: first, that nine of the island section preparations developed polyuria, though the pituitary and hypothalamus were present; second, that in those animals which did not, the completeness of the lower transection was not demonstrated. With regard to the first difficulty, the impossibility of evaluating the importance of incidental damage wrought by the operation should be pointed out. When the double section was used, the upper one may sometimes have sloped so low as to damage nuclei essential for normal water balance. If control over water balance is normally exercised through a pituitary hormone, interference with its distribution would be virtually equivalent to ablation of the pituitary body. The upper section certainly crossed the third ventricle, and may have left it gaping widely. It may have altered the circulatory rate in the pituitary or hypothalamus. By either Keller's or Bazett and Penfield's method, a clot could have obstructed the aqueduct. Each of these contingencies interrupts one of the routes which have been suggested as possible avenues for the chemical influence from the pituitary. Thus there are many reasons why the essential structures, though still remaining, might be functionally useless. Under these conditions positive results,—the twenty-one cases in which the pituitary region did seem to prevent polyuria,—seem more convincing than the negative nine.

As for the completeness of the lower transection, this was admittedly problematical. We have excluded from the report those few cats in which autopsy, or the partial development of self temperature control, demonstrated its incompleteness. That completeness was crucial for the value of the experiment was constantly borne in mind at operation. We estimate that the number of cases in which it failed was relatively small.

The conclusion from the island section is thus drawn with less assurance than the two earlier conclusions. In thirteen cases the mere presence of the isolated pituitary and hypothalamus did prevent polyuria. It also seemed to make possible adequate chloride excretion and a fairly stable condition of health. Nevertheless, in seventeen other cases, life was short and chloride output low. Nine of these showed peaks of polyuria as high as did those cats which lacked pituitary and hypothalamus. A humoral control is compatible with the results in group 3, but not proved by them.

SUMMARY

Without attempting to solve the localization problem, we have studied the function of the pituitary and hypothalamus in regulating water and chloride excretion in chronic decerebrate cats, which were given 200 cc. of milk a day.

1. When the decerebration was high, so that the pituitary and hypothalamus remained, urine and chloride excretion were small, and life was short; but the significance of this result is complicated by the extreme muscular activity of this type of preparation.

2. When the decerebration was low, urine excretion was very large, but chloride excretion and length of life were about as small as in group 1. It is concluded that the polyuria which results in these cats is due to the removal of the pituitary and adjacent structures rather than to any irritation; and that polydipsia can play no part in the origin of this polyuria.

3. When the decerebration was low, but the pituitary and hypothalamus were left in place as an island of nervous tissue, some animals survived a considerable time, and approached the normal in water and chloride excretion. The remainder varied between the limits of group 1 and group 2. Equivocal evidence for the humoral theory is furnished by these results.

It is suggested that survival in decerebrate animals may depend in part upon their ability to keep themselves in chloride balance, and this in turn upon the intactness of the hypophysis and hypothalamus.

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STUDIES ON WATER METABOLISM IN NORMAL AND HYPOPHYSECTOMIZED FROGS

MILDRED E. JONES AND F. R. STEGGERDA

From the Department of Physiology, University of Illinois, Urbana

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The function of the pituitary gland in regulating the melanophore reactions has been extensively studied and adequately reviewed by Hogben and Slome (1), Parker (2), and others. It has also been shown by Heller (3), Novelli (4), Dietel (5), Steggerda (6), and Steggerda and Essex (7) that when frogs are injected with posterior pituitary products, they increase markedly in weight due to uptake of water through the skin. Little is yet known, however, about the exact nature of this increased permeability of the skin resulting from the injections. This led to an investigation to determine if the pituitary gland might also govern the mechanism for the uptake of water following injections of pituitrin. The problem was studied by comparing the weight changes in normal and hypophysectomized frogs after pituitary injections.

The operation for removal of the hypophysis was similar to that described by Hogben (8), with this difference, that the gland was burned out instead of being removed by suction. All of the frogs showed the paleness characteristic of hypophysectomy. The criterion for the successful removal of the gland was that the frogs should remain pale on a dark background and should show no difference in body weight from the control animals over a period of 3 to 4 days. In certain cases where the frogs showed symptoms of brain injury, there was a marked increase in weight for a few days following the operation, after which time they died.

After enough frogs had been satisfactorily hypophysectomized, experiments were carried out to compare the effects of pituitrin (Parke-Davis) extracts on weight changes in normal and hypophysectomized frogs. Since these experiments were concerned with water interchanges as measured by weight, the frogs were kept nearly submerged in tap water for at least 10 to 12 hours before each experiment. At the time of the experiment, the frogs were removed separately from the water, dried with gauze as uniformly as possible, and weighed on a beam balance accurate to 0.1 gram. Then 11 hypophysectomized frogs (operated from 1–4 weeks previously) and 10 normal frogs were injected with obstetrical pituitrin, the dose being 0.1 cc. per 10 grams of frog weight. After the injections

all the frogs were replaced in the water and weighed at hour intervals for a period of six hours.

The results of these experiments show that when frogs hypophysectomized one to four weeks previous to the experiment are injected with the average dose of pituitrin, they show no sign of weight change other than that of uninjected normal control frogs. On the other hand, when normal frogs in the same water with the hypophysectomized frogs are injected with the same dose of pituitrin, they increase more than 18 per cent within

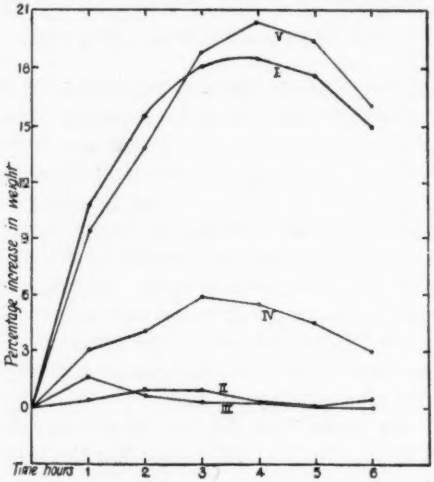

Fig. 1. Weight changes in normal and hypophysectomized frogs following injections of pituitrin.

Curve I. Average weight change of 10 normal frogs after injection of pituitrin.

Curve II. Average weight change of 11 hypophysectomized frogs injected with pituitrin (1–4 weeks after operation).

Curve III. Average weight change of 8 normal uninjected frogs.

Curve IV. Average weight change of 10 hypophysectomized frogs injected with pituitrin (1–4 days after operation).

Curve V. Average weight change of 5 frogs with cerebral hemispheres injured proximal to the pituitary gland, injected with pituitrin.

Note: The dose of pituitrin in all cases was 0.1 cc. per 10 grams of body weight.

four hours, and then gradually return to normal weight. This fact, we feel, clearly indicates that the presence of the pituitary gland in the frog is necessary in order that the pituitrin may cause some change in the skin which allows a greater absorption of water.

These results led us to inquire into the effects of pituitrin injections on weight changes in frogs shortly after the operation. A series of 10 frogs

hypophysectomized 1 to 4 days previously were injected with pituitrin (0.1 cc. per 10 gm. wt.). The average curve for this experiment indicates a slight but definite increase in weight, 6 per cent within three hours, and a gradual return to normal. This might mean that there are still some vestiges of pituitary products in the body of the frog, which continue to function in controlling the uptake of water after pituitary injections. This finding is quite in agreement with those of Heller (3) and Steggerda and Freedman (9), who report that the ability of frogs to respond to pituitrin is decreased after decapitation.

That the effects obtained are related to the removal of the pituitary gland, and not to injury of certain parts of the brain, was shown by experiments in which the cerebral hemispheres were injured just anterior to the hypophyseal region, and by others in which the brain had been destroyed with a pithing needle in the usual way. Although it is possible that pithing may injure the pituitary gland, the effects are not significant because when these frogs are injected with pituitrin, they show increases in weight very similar to those of normal frogs injected with pituitrin.

Each of the four experiments described was controlled by two normal uninjected frogs kept in the same water and weighed at the same time as the experimental frogs. Thus the control curve represents an average of eight frogs.

The question arose as to whether or not the dose used in the hypophysectomized animal was concentrated enough. This was tested by giving a double dose of pituitrin to 4 frogs hypophysectomized 2 to 4 days previously and to 3 normal frogs. It was found that the hypophysectomized animals showed no additional change in weight, whereas the normal frogs increased 32 per cent in body weight, nearly double the increase attained after a single dose. Repetition of a single dose of pituitrin to the same group of hypophysectomized frogs on two successive days was without effect, indicating the absence of any summation effects.

CONCLUSIONS

1. Hypophysectomized frogs, 1 to 4 weeks after operation, show no weight increase when injected with pituitrin. Control frogs similarly injected increase 18 per cent.
2. Hypophysectomized frogs, when injected with pituitrin 1 to 4 days after operation, show a slight increase in weight.
3. Cerebral injury does not alter the ability of pituitrin to produce weight changes.
4. The presence of the pituitary gland in the frog appears to be essential for the pituitrin to bring about the characteristic increase.

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THE PERMEABILITY OF FROG CAPILLARIES TO PROTEIN

RUTH E. CONKLIN

From the Department of Physiology, Vassar College, Poughkeepsie, N. Y.

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The marked permeability to protein of the capillaries of the frog's skin was first demonstrated by Churchill, Nakazawa and Drinker (1927). The plasma proteins are all found in the lymph from subcutaneous sacs, and other colloidal substances such as human hemoglobin and certain dyes pass easily through the capillary endothelium (Conklin, 1930b). Previous experiments, while they showed ease of passage from blood to lymph, indicated that normally this permeability was a one-way phenomenon, and that the protein, once out of the blood, must return to it by the lymphatic route. This left open the question whether the endothelial wall really prevented the return of protein to the blood, or whether it was largely a matter of a diffusion gradient. If, for instance, the concentration of protein in the blood as compared with the lymph could be reversed, the protein might then enter the blood through the capillary endothelium instead of by the circuitous lymphatic route.

Method. To test this hypothesis two series of experiments were undertaken in which the same method was followed as in earlier experiments, i.e., the washing out of plasma proteins by the injection of Ringer's solution into the ventral abdominal vein of curarized frogs, and the collection of lymph from drainage cannulas placed under the skin. Since curare stops the lymph hearts it would be impossible for protein in the lymph to return to the blood by the usual passage through the lymph hearts.

Series A. With the blood reduced in protein, as shown by refractometric determinations of the plasma and lymph, subcutaneous injections were made of frog plasma or normal horse serum. Three hours later a blood sample was taken from the heart and the plasma proteins determined refractometrically.

Series B. The serological method was used to detect the presence of horse serum in the blood. Horse-immune rabbit serum giving a titer of 1:16,000 was prepared for this purpose. A number of experiments were done first with Rana pipiens, but many were unsuccessful because of the difficulty of obtaining enough blood at the end of the experiment for a precipitin test. Additional experiments were, therefore, carried out on Rana catesbiana.

TABLE 1

EXPERIMENT NUMBER	LYMPH, PER CENT PROTEIN DURING BLOOD DEPLETION				INJECTION		PLASMA, PER CENT PROTEIN	
	½ hr.	1 hr.	1½ hr.	2 hrs.	Amount	Per cent protein	Before injection	After injection
1	2.25		2.47	1.92	1 cc. horse serum	5.96	1.30	1.80
2	3.27		2.03		1 cc. horse serum	5.96	1.74	2.74
3		2.23	1.58		1 cc. horse serum	5.96	0.90	2.06
4	3.77	3.71	2.61	1.85	1 cc. frog plasma	3.94	0.23	2.34
5	4.21	3.39	2.74	2.05	1 cc. frog plasma	3.72	0.63	1.84
6	3.61	3.53	2.65	2.05	1 cc. frog plasma	3.94	0.49	1.60
7	4.21	3.55	2.96	2.39	None, control		0.97	2.48
8	0.83	1.06	0.94	0.83	None, control		0.45	0.69

TABLE 2

Frog serum tests with horse-immune rabbit serum

EXPERIMENT NUMBER	SPECIES AND TREATMENT	PRECIPITIN TEST AFTER 1 HOUR						
		Undiluted frog serum	1/10	1/100	1/500	1/1000	1/2000	1/4000
	Experimental							
10	R. pipiens	++	+	–	–	–	–	–
11	Curare	++	+	–	–	–	–	–
12	10 cc. Ringer's solution	+++	++	+	–	–	–	–
13	1 cc. horse serum	++	+	–	–	–	–	–
14		+++	++	+	–	–	–	–
15	R. catesbiana	++	+	–	–	–	–	–
16	Curare	+++	++	+	±	–	–	–
17	100 cc. Ringer's solution	+++	++	+	–	–	–	–
18	10 cc. horse serum	++	+	–	–	–	–	–
19		+++	++	++	+	–	–	–
	Controls							
1	R. pipiens (2)	–	–	–	–	–	–	–
	R. catesbiana	–	–	–	–	–	–	–
	No experimental procedure							
2	R. pipiens	+	±	–	–	–	–	–
	R. catesbiana	–	–	–	–	–	–	–
	Curare and horse serum; no blood depletion							
3	R. pipiens (2)	+++	++	+	–	–	–	–
	R. catesbiana	++++	+++	+++	++	+	–	–
	Horse serum only. No curare nor blood depletion							

The procedure consisted in curarizing the frog, then cannulating the ventral abdominal vein and injecting through it oxygenated Ringer's solution. In Rana pipiens, 10 cc. were injected over a period of two hours. In Rana catesbiana, which weighs approximately ten times as much as the leopard frog, 100 cc. were injected over the same length of time. Several cannulas were tied under the skin and lymph was drained from them. It is easy by this method to wash much of the protein out of the blood with only a slight rise in blood pressure (Conklin, 1930b), and there is the distinct advantage of avoiding withdrawal of blood as in plasmapheresis, a procedure very difficult in frogs owing to their small blood volume.

At the end of the injection period normal horse serum (1 cc. in R. pipiens, 10 cc. in R. catesbiana) was injected subcutaneously in the thighs, and the frog was left for a period of one, or usually two hours. A sample of blood was then withdrawn from the left aorta, allowed to clot, and the serum tested with horse-immune rabbit serum.

Results and discussion. *Series A*. The results are given in table 1. It will be seen that there was always an increase in plasma protein, indicating probable absorption through the capillaries. Since the control frogs, which were given no injection, also showed an increase, the method was not decisive in proving the added protein to have entered from the lymph sacs. Regeneration may have occurred very rapidly from the liver or elsewhere.

Series B. The serological findings are summarized in table 2. In all cases horse serum was found in the blood of curarized frogs. An inspection at the end of the experiment always showed that the lymph hearts were not beating, so that the horse serum must have entered the blood through the capillary endothelium. In the control experiments it is evident that in curarized frogs whose proteins have not been depleted the horse serum gets through the capillaries in very small amounts if at all, whereas in non-curarized frogs it can enter by the usual lymphatic route.

It seems evident that the normal, one-way permeability to protein of the skin vessels of the frog is due to a diffusion gradient rather than to any obstacle imposed by the endothelial wall. This same condition has been demonstrated in the dog by Field and Drinker (1931). Due to the enormously greater production of lymph in the frog than in mammals (Isayama, 1924a and b; Conklin 1930a), and to the permanent excess of capillary blood pressure over the osmotic pressure of the plasma colloids (Churchill, Nakazawa and Drinker, 1927) creating a steady hydrostatic flow outwards, there is even less chance in the frog than in mammals for reverse permeability to proteins under ordinary conditions. In spite of these factors it is plain that there can be passage of proteins into the capillaries, when the plasma protein content is depleted.

SUMMARY

1. The capillaries of the frog's skin, though highly permeable to protein, normally do not admit protein molecules from the lymph spaces.

2. When the plasma proteins have been depleted, horse serum, injected into subcutaneous lymph spaces in curarized frogs, will pass through the capillary endothelium and may be recognized in the blood by serological means.

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